Identifying Signaling Pathways Mediating Filamentous Growth in *Saccharomyces Cerevisiae*

by

Cole A Johnson

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Molecular, Cellular, and Developmental Biology)
in the University of Michigan
2013

Doctoral Committee:

Associate Professor Anuj Kumar, Chair
Associate Professor Matthew R. Chapman
Professor Daniel J. Klionsky
Assistant Professor Dongxiao Zhu, Wayne State University

Dedication

To my loving wife,

A Dedication to My Wife
by: T.S. Eliot

To whom I owe the leaping delight
That quickens my senses in our wakingtime
And the rhythm that governs the repose of our sleepingtime,
The breathing in unison

Of lovers whose bodies smell of each other
Who think the same thoughts without need of speech
And babble the same speech without need of meaning

No peevish winter wind shall chill
No sullen tropic sun shall wither
The rose-garden which is ours and ours only

Bu this dedication is for others to read:
These are private words addressed to you in public

Acknowledgements

I would like to extend my sincerest gratitude to those who made this dissertation possible. To my advisor and friend, Dr. Anuj Kumar, thank you for your understanding and mentorship during my tenure at U of M. You kindly accepted me "slightly used" into your lab and continually instilled the virtues of mixing solid research with good music and yummy food. I imagine I still have a lot to learn about good music…

I would also like to thank my committee members, Dr. Matthew R. Chapmen, Dr. Daniel J. Klionsky, and Dr. Dongxiao Zhu, for always being there to provide valuable contributions to my research along the way.

It is especially important that I acknowledge the members of Kumar lab, both those we've lost and those we've gained. Each of you have helped me to grow personally in your own way; whether it be an optimistically cynical outlook on life, a renewed appreciation for the struggles of women in science, an increased basal blood alcohol content, or the ability to live life vicariously through your "Why not?"-motivated actions, graduate school has been more enjoyable because of you.

Finally, I would like to explicitly thank Mary Carr, without whose help I'd probably be on the streets, along with the other members of the MCDB faculty and staff who have supported me along the way.

Go Blue!

Chapter II of this dissertation has been published as:

Judeh, T., Johnson, C., Kumar, A., Zhu, D. (2013). TEAK: topology enrichment analysis framework for detecting activated biological subpathways. Nucleic Acid Res 41(3), 1425-37.

Chapter III of this dissertation has been published as:

Johnson, C., Kweon, H.K., Sheidy, D., Shively C.A., Mellacheruvu, D., Nesvizhskii, A., Andrews, P.C., Kumar, A. (2013). The Yeast Sks1p Kinase Signaling Network Regulates Pseudohyphal Growth and Glucose Response. s*ubmitted.*

Other publications:

Bharucha, N., Charbrier-Rosello, Y., Xu, T., Johnson, C., Sobczynski, S., Song, Q., Dobry, C., Echwahl, M., Anderson, C., Benjamin, A., Kumar, A., Krysan, D. (2011). A Large-Scale Complex Haploinsufficiency-Based Genetic Interaction Screen in Candida Albicans: Analysis of the RAM Network during Morphogenesis. PLoS Genetics 7(4), e1002058.

Xu, T., Johnson, C., Gestwicki, J.E., Kumar, A. (2010). Conditionally controlling nuclear trafficking in yeast by chemical-induced protein dimerization. Nat Protocols 5(11), 1831-43.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

An introduction to yeast

1.1. A brief history of yeast research

Humankind has leveraged yeast in the process of fermentation to produce alcoholic beverages and to leaven bread since ancient times. In fact, yeast is likely the oldest, domesticated, industrial microorganism with archaeological records indicating its use for brewing in Sumeria and Babylonia over 7,000 years ago (1–3), actually predating the development of a written language. Even the species name of the most commonly used yeast, *Saccharomyces cerevisiae* also known as baker's yeast, demonstrates the historical significance of this organism in producing alcoholic beverages (*cervisia*, *cervesia*, or *cerevisia* are Latin for beer) (4).

Though yeast was first observed by van Leeuwenhoek in 1680 (**Figure 1.1)**, it was another century and half before improvements in microscopy allowed the scientists Cagniard-Latour , Kützing, and Schwann to independently define yeast as a living organism that grows by budding, ferments sugar to produce ethanol, requires nitrogen-based substances, and has an estimated diameter of 6-9 μm (4). Despite these observations, the idea that yeast was a living organism was heavily challenged by leading chemists of the day, and their influence negatively impacted the development of microbiology. It took nearly another two decades before Louis Pasteur affirmed the role of yeast in alcoholic fermentation and undisputedly established yeast as living.

Nevertheless, the early study of alcoholic fermentation produced vast amounts of scientific knowledge and, one could argue, birthed the modern life sciences. Advancements made while studying fermentation spurred the development of biochemistry and microbiology. For instance, the word enzyme actually has its origin from the Greek *en* (εν) meaning 'in' and *zymi* (ζυμι) meaning "leaven" or "yeast" and used to refer to yeast, dough, or as a root for words associated with beer and fermentation (1, 2). This now frequented term was actually created to identify

compounds derived from yeast that can ferment sugar through observations that date back to the earlier studies of Pasteur (5). Since then, the study of yeast fermentation has contributed greatly to our understanding of microbial physiology, microbial pathology, and enzymology, eventually leading to the acceptance of enzymes as the drivers of cellular metabolism after the long controversy that began with the discovery of yeast being a living organism by Cagniard-Latour, Kützing, and Schwann (6, 7). Additionally, *S. cerevisiae* and other yeasts are responsible for a vast majority of industrial and medical applications beneficial to human life (8) (**Figure 1.2**). Modern applications of yeast include fermented foods and beverages, chemicals (reviewed in (9, 10)), nutraceuticals, pharmaceuticals such as the Hepatitis B vaccine (1, 11), and biofuels (12).

1.2. Yeast as a model organism

The unique properties of the yeast *S. cerevisiae* extend beyond those humans have exploited for thousands of years in baking and brewing; they also make it an exceptional organism for research. Initially introduced as an experimental organism in the mid-1930's (13), yeast rapidly became the workhorse for modern cell and molecular biology due to several advantageous qualities.

First, *S. cerevisiae* cells are unicellular but eukaryotic, non-pathogenic, have minimal nutritional requirements, and reproduce by budding every 90 minutes. This means it can easily be maintained in a laboratory setting, and experiments typically proceed more rapidly than with other eukaryotic organisms.

Yeast also has remarkably tractable genetics in that DNA can be easily and precisely added or removed from its genome via homologous recombination. Driven by the discovery of Gerald Fink's group that yeast could be transformed with foreign DNA (14) and the simultaneous development of an *E. coli*/*S. cerevisiae* shuttle vector by Jean Beggs (15), genetic engineering quickly become the crux of yeast's popularity and success as an experimental system. In fact, the effectiveness at which the yeast genome can be manipulated has led to the popular colloquialism "the awesome power of yeast genetics" (16). Moreover, while most wild strains of *S. cerevisiae* are diploid (17), lab strains can typically grow as stable haploids, which further facilitates genetic manipulation. The haploid yeast genome is just over 12 million base pairs in length, consisting of 16 chromosomes ranging from 230,000bp to 2,352,000bp in size

(18). The haploid *S. cerevisiae* genome was the first eukaryotic genome to be successfully sequenced (19), which provided an unprecedented view of the nearly 5800 protein-coding genes (4% of which have introns) comprising 70% of the total genome. Consequently, the yeast genome has become the premier eukaryotic genome (20).

Yeast has substantially contributed to our understanding of eukaryotic biology due to its easy manipulation, the elegance of its genetics, and the availability of both a complete genome sequence and a vast arsenal of molecular biological tools (see below). For example, most of our knowledge regarding the cell-division cycle is a product of yeast experimental research (21). This success is also due to the remarkable biological conservation between yeast and other eukaryotes. Yeast cells divide in a similar manner to human cells and share many other basic cellular processes such as cell cycle progression, nucleic acid transcription and translation, protein targeting and secretion, and cytoskeletal structures. Moreover, yeast share a high number of gene orthologs with higher eukaryotes; almost 31% of putative yeast protein-encoding genes have homology to mammalian protein sequences (22). Nearly 100 yeast genes (~17%) are members of orthologous gene families associated with human disease (23). The mammalian homolog for many of these genes is functional in yeast and complements the yeast deletion mutant. This suggests these diseases are a consequence of disrupting the basic cellular processes shared amongst eukaryotes, such as DNA repair, cell division, or regulation of gene expression. Therefore, yeast can be leveraged to investigate functional relationships involving these genes, as well as others, to better understand human disease states (24, 25) including cancer (26–28) and neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's (29, 30). *S. cerevisiae* is also an excellent model for the study of diverse plant and human pathogenic fungi (31). The signaling pathways and associated genes that regulate mating and virulence in the plant fungal pathogens such as *Ustilago maydis* and the human fungal pathogens *Cryptococcus neoformans* and *Candida albicans* are highly conserved in yeast (32). Ergo, much of our current knowledge of the genetic pathways controlling virulence in fungal pathogens, specifically those involved in filamentous growth, has been determined by studies in *S. cerevisiae* (33, 34).

1.3. Yeast genomics

Not long after the yeast genome was published, another broad international initiative began to generate a genome-wide, yeast deletion collection (35, 36) in which nearly every yeast

open reading frame (ORF) was replaced with a bar-coded drug-resistance gene. Thus, each deletion is individually identifiable in screens of the entire mutant library. Around this same time, another yeast mutant collection was generated by exploiting transposons, mobile genetic elements that can be used to disrupt genes in a non-targeted fashion, to randomly mutate the yeast genome (37, 38). These mutant libraries and their derivatives have been successfully exploited in a vast array of genome-scale experiments allowing researchers to implement many novel screens for mutant phenotypes under more than 100 experimental conditions; these studies allowed for the assignment of over 5,000 novel phenotypic traits to yeast genes (reviewed in (39)).

Many other useful yeast mutant libraries have been constructed for the analysis of gene function. A library of yeast genes C-terminally-tagged with green fluorescent protein (GFP) was created to visualize the subcellular localization and interactions of most of the proteins in yeast (40). Similarly, Ghaemmaghami *et al* generated a yeast fusion library where each ORF was chromosomally tagged with a high-affinity tandem affinity purification (TAP) tag (41), which allows immunodetection and immunopurification of the entire yeast proteome. Yet another collection was designed where each of its 5,573 total yeast strains carries a multicopy plasmid bearing a unique yeast ORF tagged C-terminally with a tandem affinity tag that includes a hemagglutination (HA) tag and driven by an inducible *GAL1* promoter (42).This collection allows the targeted over-expression of most yeast genes and was recently used in a genome-wide study to identify novel regulators of filamentous growth in *S. cerevisiae* (43).

Collectively, these yeast collections offer a unique and powerful toolkit for large-scale investigations into the relationship between genotype and phenotype. These numerous tools and ease of experimentation in yeast ensures that it will continue to be a very popular test bed for the development of novel high-throughput technologies. And though the collections presented above have significantly contributed to the advancement of functional genomics, they are far from exhaustive catalogs of yeast collections. Numerous yeast culture collections exist bearing thousands of strains across multiple species (reviewed in (44)).

1.3.1. Gene expression analysis

The arrival of a completed genome also impelled considerable technology development toward the study of genome-wide gene expression, specifically the characterizing of the

transcriptome, the complete set and quantity of transcripts in a cell under a particular condition. In 1995, Schena and colleagues reported DNA microarrays as an ideal tool for the systematic, high-throughput, and quantitative investigation of gene expression on a large scale (45). In this method (**Figure 1.3**), cDNA products are amplified by PCR and subsequently attached to a solid surface, typically a glass slide, in an organized fashion. Two mRNA samples purified from an experimental cell population and a control cell population are then transcribed into cDNA and fluorescently labeled, each with its own fluorescent dye. The cDNA samples are then mixed and used to query the cDNA products affixed to the glass surface. Leveraging the naturally ability of complementary DNA to hybridize, the mixed cDNA products are washed over the microarray and then detected via laser scanning, such that the bound cDNA samples emit a detectable fluorescent signal that indicates the expression of the particular gene located at that position on the glass surface as measured by the ratio of the two fluorescent signals (experimental/control). Many different variations of DNA microarray technologies have since been developed to measure many genomic features in addition to gene expression, including genotyping for point mutations, single nucleotide polymorphisms (SNPs), and DNA-methylation (reviewed in (46)) as well as for the clinical detection of infectious diseases (47).

In yeast, microarray approaches have been used extensively to characterize the transcriptome upon exposure of cells to numerous conditions and stresses (48, 49). DNA microarrays in yeast have also led to improvements in the effectiveness of industrial yeast strains (50), in anticancer drug therapies (51), and in our understanding of fungal pathogenicity (52, 53).

As described above, microarray studies are a preferred tool for large-scale gene expression experiments. However, robust experimentation necessitates validation of at least a subset of the relative gene expression changes discovered via DNA microarray analysis. In addition, whole-genome gene expression is generally not necessary when the gene or pathway is already known. In both of these situations, the data collection or validation can begin with quantitative real-time PCR (qRT-PCR). Since its development in the early 90's (54), qRT-PCR has permitted sensitive detection and quantification of specific DNA sequences (reviewed in (55, 56)). The procedure exploits the general principle of polymerase chain reaction (PCR) to fluorescently detect each copy of DNA as it is amplified from a provided cDNA template in real time. As each PCR cycle produces an increased level of total double-stranded DNA, the rate of

amplification can be used to calculate the relative measurement of the initially supplied cDNA template.

qRT-PCR is a standard tool used commonly to detect and/or validate changes in gene expression, such as identifying transcriptional changes of genes regulating fungal pathogenicity (57, 58). However, it can also be used to improve the diagnosis of cancer and infectious diseases (59, 60) or for the detection of bacterial, fungal, or viral pathogens (61, 62).

Hybridization-based transcriptome studies offer a high-throughput and relatively inexpensive approach for elucidating the relationship between genotype and phenotype. Yet, few technologies have revolutionized the biological sciences as much as the development of massively parallel, next-generation DNA sequencing. For a review on next-generation sequencing technologies refer to (63, 64). These deep-sequencing technologies allow entire genomes to be sequenced in a very short time, in parallel, and with an amazing level of sensitivity and accuracy. Gene expression analysis using sequence-based approaches had been exploited with low-throughput technologies like sequencing EST libraries (65) and even with more high-throughput options like serial analysis of gene expression (SAGE) (66) and massively parallel signature sequencing (MPSS) (67), but these technologies were limited by experimental design and available technology. Next-generation sequencing spurred the development of a novel high-throughput sequencing technique that allowed both mapping and quantifying transcriptomes called RNA-seq (68) (**Figure 1.4**). By this methodology, a population of RNA is converted to a library of cDNA fragments with adaptors attached to one or both ends. The cDNA library is then submitted to deep sequencing using, in principle, any available high-throughput sequencing platform. RNA-seq can accurately determine the expression levels of specific genes, reveal sequence variations (SNPs), identify novel transcripts and isoforms, and characterize non-coding RNAs (69, 70). Also, unlike microarrays, RNA-seq can be used on organisms without a sequenced genome.

RNA-seq has enabled transcriptomics to proceed at an unprecedented rate and level of precision (71) and full transcriptomes have been sequenced for numerous organisms including human, mouse, yeast, worm, and several plants across various conditions (72). Also, unsurprisingly, this technology was developed and first implemented in *S. cerevisiae* (73).

1.3.2. Protein interaction analysis

A predictive understanding of the rate, quantity, and form in which genes are transcribed still fails in defining the full functional relationship between genotype and phenotype because it lacks the characterization of proteins and their interactions, which are largely responsible for the execution and control of most cellular processes. One of the earliest tools for investigating protein-protein interactions, the yeast two-hybrid assay, was developed in yeast over two decades ago and is still an important contributor in the arena (74). In this system, the *S. cerevisiae GAL4* protein is split into two functional domains: a DNA binding domain, which binds to a specific genomic upstream activation sequence (UAS), and transcriptional activation domain, that activates transcription of a reporter gene, typically the *E. coli lacZ* gene, downstream of the UAS (**Figure 1.5**). The protein of interest (the bait) is fused to the GAL4 DNA binding domain, while the transcription activation domain is typically attached to different putative interaction proteins (the prey) within a cloned library. Activation of the reporter gene only occurs when the bait and prey proteins interact, which positions the activation domain in the position to activate *lacZ* transcription.

Numerous large scale studies have been performed with this or variations of this yeast-centric system (75), including novel drug-protein interaction studies for the development of new therapeutics (76, 77). For a more thorough review of the yeast two-hybrid methods and its applications see (78).

Despite being widely used to uncover a vast number of interactions, this method has also received a fair amount of criticism typically for its high rate of false positives (79). A more reliable approach leverages the versatility and power of mass spectrometry for the comprehensive characterization of proteomes (80). Modern mass spectrometry-based proteomics offers an analytical platform capable of quantifying protein abundance, identifying post-translation modifications, and revealing members of protein complexes on a large-scale (81). In general, the first step in any proteomics experiment is sample lysis and protein extraction followed by proteolysis of the extracted proteins into peptides. The peptides can then be enriched, for phosphopeptides or other post-translational modifications, before being submitted to liquid chromatography coupled to mass spectrometry (LC-MS/MS), where the peptides are identified, statistically validated, and assembled into proteins (**Figure 1.6**).

Early uses of mass spectrometry proteomics generated vast amounts of proteomic data (82), including characterizing protein interactions across nearly the entire yeast proteome (83) and a significant portion of the human proteome (84) as well as identifying biomarkers of cancer progression (85). A major innovation to the quantification of LC-MS/MS is the technique of stable-isotope dilution. This approach leverages the fact that a stable isotope-labeled peptide is chemically identical to its native equivalent and, as such, should behave identically in downstream analyses. Mass spectrometers can detect the mass differences between the peptides permitting accurate quantification of unlabeled to labeled peptides (86, 87). While many variations of stable-isotope tagging have been introduced (reviewed in (88)), a particularly attractive yeast method called 'stable-isotope labeling with amino acids in cell culture', or SILAC, offers a simple and effective means to label all peptides for proteomic analysis (**Figure 1.7**). In this method (89), labeled, essential amino acids are added to amino acid-deficient media. Cells grown in the SILAC media metabolically incorporate the labeled amino acids into their proteins as they are produced. This method has been used for multiple large-scale studies, including a proteomic comparison of haploid and diploid yeast (90) and a genome-wide analysis of protein phosphorylation in mammalian cells (91). However, the cost and time associated with creating the cell lines often negates the value of information provided, though the ease of yeast genetic manipulation make it an exceptional organism for this methodology.

In recent years, MS-based proteomics have experienced vast improvements in instrumentation, computational methods, and sample preparation (92) that have greatly increased the depth of proteome coverage and sample throughput. Indeed, a single quantitative MS-based phosphoproteomic study can now identify more than 30,000 phosphosites (93). As the life sciences shifts toward systems level biology, it is becoming ever important to obtain a more holistic understanding of the component parts regulating the diverse physiological processes and their response to environmental changes. Mass spectrometry proteomics offers a powerful analytical tool for investigating one component modulating these processes, the protein interaction networks, on a large scale. Undoubtedly, yeast will continue to provide an excellent test bed for such technological developments and large-scale studies.

1.3.3. Data storage, analysis, and integration

With the development and increasing use of high-throughput -omic technologies comes an unprecedented increase in the production of experimental data. Success in the life sciences will require the integration of these large-scale, high-dimensional data sets to attain a predictive understanding of living systems. To achieve such a vast and integrated system, several infrastructures are believed necessary, which include data, communication, computational, and human infrastructures (94). First, biology is an information-driven science and, as such, progress rests on the ability to produce, share, and freely access new data (20). The data infrastructure is housed in the numerous online databases that were developed in the rise of the -omics era. These resources store vast amounts of high-throughput data including those from genomic, proteomic, and metabolomic studies (20, 95). These databases can even be organism specific, for instance anyone seeking information on just about any aspect of *S. cerevisiae* biology has access to the amazing community resource: the Saccharomyces genome Database (SGD; http://www.yeastgenome.org).

Recently, there has been more focus on transitioning these data warehouses into more unified, functional, and relational sets of data that will better promote genotype-to-phenotype analyses (96) and improve clinical diagnosis (97). Critical to the development of these advanced electronic resources is the standardization of data structures so that the myriad of existing data sources are more interoperable (98). For instance, if an investigator is attempting to integrate data from two distinct repositories to achieve novel biological insights it is important, but uncommon, that the data from the two sources are syntactically and semantically compatible with each other (94). The transfer of functional information between and among databases requires not only that the data are formatted similarly, but also that the functional information embodied in the data is annotated using an interoperable terminology, called an ontology. The goal is to be able to describe gene and protein function in a manner that is biologically relevant, yet compatible with computational methods. Standards for many types of functional –omics data have been created, the most successful and cross-functional controlled vocabulary being that created by the Gene Ontology Consortium (99). GO annotations describe gene products in terms of their associated biological processes, cellular components, and molecular functions in a species-independent manner permitting functional information to be easily connected to and through other databases. Notably, most of the GO annotations were derived from yeast-based

experiments (79). Many other ontologies are available and the Open Biological Ontologies (OBO) Foundry (http://www.obofoundry.org) site was generated to coordinate, organize, and maintain both the new and over 60 existing ontologies (100). Continued standardizing efforts will hopefully promote the efficient integration of experimental data into existing systems of genotype and phenotype databases to improve our ability to analyze and understand complex biological systems.

Efficient and structured data storage would be pointless without the computational ability for analysis. However, the powerful combination of computational and experimental research is only beneficial so long as those computational methods can keep up with the ever-increasing size and heterogeneity of experimental data. Fortunately, novel algorithms continue to be developed that increase our ability to integrate and analyze –omics data (reviewed in (101)). Since most high-throughput data typically yield a list of differentially expressed genes or proteins, one popular approach for extracting meaningful biological insight from this list is to simplify the analysis by grouping the genes or proteins that function in the same pathways during analysis, known as pathway enrichment. This approach reduces the complexity of and increases the explanatory power in predicting the gene regulatory network responsible for the observed gene expression (see (102) for an excellent review of pathway analysis).

Notably, gene expression data from yeast has been a developmental sandbox for many platforms designed to infer gene networks (103). Many of the bioinformatic approaches developed in yeast have been successfully applied to data on humans (104), e.g. (105, 106), as well as other model organisms (107). More recent algorithms have even been validated in yeast to predict regulatory sub-pathways from gene expression data (108). For an overview of databases and repositories that offer tools for the integrative analysis and modeling of data generated by the different -omics approaches in yeast see (109).

1.4. Fungal pathogens and filamentous growth

Nosocomial fungal infections, those that are contracted while under hospital care, are a significant cause of morbidity and mortality, particularly in high-risk individuals such as those that are immunocompromised or in the ICU. Invasive fungal infections (IFIs) in these populations nearly triple the mortality rate and drastically increase the length of hospitalization resulting in an estimated $2 billion in additional costs (110, 111). In fact, the top ten fungal

diseases are responsible for nearly as many annual deaths as tuberculosis or malaria (112). To make matters worse, the incidence of these infections continues to increase (113). Many fungal species are responsible for IFIs, but the most reported fungal-associated fatalities are due to species from the genera *Cryptococcus*, *Candida*, *Aspergillus*, or *Pneumocystis*. Most of these fungi also have the ability to switch between a cellular yeast form and a filamentous form in response to environmental stimuli and, in many species, this dimorphic transition is linked to virulence (114–116). Notably, the budding yeast *S. cerevisiae* can also undergo the dimorphic transition to filamentous form in response to environmental signals (117). In addition, *S. cerevisiae* is a close relative of the *Candida* species (118) and, though uncommon, it can become pathogenic (119–121). These traits together with those previously described make *S. cerevisiae* an ideal model system for elucidating the mechanisms controlling fungal pathogenicity (122).

1.4.1. Filamentous growth in *S. cerevisiae*

The fact that certain strains of the budding yeast *S. cerevisiae* are a capable of filamentous growth was only recently re-discovered by the lab of Gerald Fink (123). Interestingly, the Fink lab later determined that most commonly used lab strains, those derived from S288c, have a non-sense mutation in *FLO8*, a gene necessary for the morphological transition to filamentous form (124). The "wild" strain background characterized by the Fink lab (strain Σ1278b or the Sigma strain) is the leading genetic background for studying filamentous growth in *S. cerevisiae*.

Filamentous growth in budding yeast is characterized by a morphological transition from single, oval-shaped cells to chains of elongated cells, called pseudohyphae, that can spread out along the surface of a solid growth substrate as well as invade the substrate (125, 126). This phenomenon is considered to be an adaptive mechanism to forage for nutrients when local resources become limited as the elongated morphology allows the cells to more effectively forage for additional nutrients (**Figure 1.8)**. Driving these morphological changes are a host of altered developmental processes, including a delay in the G2-M cell-cycle transition that produces elongated cell morphology (127), a switch from axial (in haploid) or bipolar (in diploid) budding pattern to a unipolar budding pattern (128), and increased cell-cell adhesion (129). Interestingly, the pathways responsible for all of these programs overlap significantly (see below).

Though both haploid and diploid yeast can transition to filamentous form, the control mechanisms and the resulting morphological changes can differ slightly between the two (34). Both cell types can be induced to form pseudohyphae by either nitrogen or glucose limitation/deprivation (34, 123, 130, 131). Similarly, it was found that short chain alcohols, such as butanol, can also trigger filamentous growth in both cell types (132). Further studies have indicated budding yeast use secreted alcohols as a quorum sensing mechanism to assess cell density and available nutrients to induce filamentous growth (133).

As previously mentioned, various aspects of filamentous growth in *S. cerevisiae* are shared with fungal pathogens, particularly *Candida albicans* and *Ustilago maydis*. There exists high conservation between the pathways and components mediating filamentous growth in budding yeast and these fungi. However, even in *S. cerevisiae*, this dimorphic switch is a complex phenotypic response that involves the integration of multiple nutritional and environmental signaling cascades to control diverse cellular processes (134).

1.4.1.1. Signaling pathways that regulate filamentous growth in *S. cerevisiae*

In yeast, there are at least four well characterized signaling pathways leading to filamentous growth: the mitogen-activated protein kinase (MAPK) pathway, the nutrient sensing cAMP-dependent protein kinase A (PKA) pathway, the sucrose non-fermentable (SNF) pathway, and the target of rapamycin (TOR) pathway. Each of these pathways, as well as other well described regulators of yeast filamentous growth, are discussed briefly below and recently reviewed in (32, 34, 117, 135).

1.4.1.1.1 The mitogen-activated protein kinase (MAPK) pathway

Mitogen-activated protein kinase (MAPK) pathways are among the most ubiquitous mechanisms of eukaryotic cell regulation. These highly conserved signaling cascades regulate diverse cellular responses to changing environmental conditions and numerous other stimuli, including cell growth, gene expression, differentiation, cell survival, and apoptosis (136). MAPK pathways consist of a triple kinase module where a MAPK kinase kinase (MKKK) phosphorylates and activates a MAPK kinase (MKK) which in turn activates a MAPK, typically via dual phosphorylation of conserved threonine and tyrosine residues(137). Activated MAPKs often translocate from the cytoplasm to the nucleus where they phosphorylate targets, e.g.

transcription factors, to modulate expression of specific genes in response to particular extracellular stimuli.

In budding yeast, five MAPK pathways have been defined: the pheromone-responsive mating pathway regulated by the MAP kinases Fus3p (essential for mating response) and Kss1p, the nutrient-responsive filamentous growth pathway regulated by MAP kinase Kss1p, the high osmolarity/glycerol pathway regulated by Hog1p, the cell wall integrity pathway controlled by MAP kinases Slt2p/Mlp1p, and the spore wall assembly pathway mediated by MAPK Smk1p (138). Three of these MAPK pathways (mating, filamentous, and osmo-regulatory) appear to share common components but regulate distinct target genes and generate disparate morphogenic responses (139)(**Figure 1.9**). Interestingly, these pathways implement several mechanisms to prevent undesirable inter-pathway cross-talk despite having overlapping sets of signaling components (140), suggesting an unappreciated specificity in these signaling networks.

In response to certain nutrient limiting conditions, e.g. low nitrogen, an extracellular signal activates the regulatory pathway responsible for filamentous growth. The protein receptors that initiate filamentous growth have not been fully identified, but recent studies indicate that the signaling mucin Msb2p (141), the integral membrane protein Opy2 (142, 143), and cell-surface regulator Sho1p (144) all function upstream of the core filamentous growth MAPK pathway. Activation of Msb2p and/or Sho1p along with other unidentified proteins stimulates the activity of the rho-like GTPase Cdc42p through the guanine nucleotide exchange factor (GEF) Cdc24p. Activated Cdc42p interacts with the p21-activated kinase (PAK) Ste20p, which in turn initiates the MAPK cascade consisting of the MAPKKK Ste11p, the MAPKK Ste7p, and the filamentous growth-specific MAPK, Kss1p (145). Interestingly, Kss1p both inhibits and stimulates filamentous growth (146). In its unphosphorylated state, Kss1p and the filamentous transcriptional regulator Ste12p associate with the filamentous growth-repressive proteins, Dig1p and Dig2p (147). Upon phosphorylation by Ste7p, Kss1p phosphorylates Ste12p, Dig1p, and Dig2p causing their dissociation. Free and activated Ste12p then induces the expression of the transcription factor *TEC1* and also binds cooperatively with Tec1p to genomic regions known as filamentous growth response elements (FREs) to activate genes involved in the filamentous response, such as *FLO11*.

1.4.1.1.2. The cAMP-dependent protein kinase A (PKA) pathway

In *Saccharomyces cerevisiae*, the cAMP-dependent PKA pathway plays coordinates numerous cellular processes such as metabolism, proliferation, stress resistance, cell growth, autophagy, and filamentous growth (148–151). In fact, the cAMP-PKA pathway is the primary means by which yeast cells respond to changes in extracellular nutrients, e.g. glucose, to regulate cell cycle progression (33). Nutrient limitation increases intracellular cAMP levels, which activates PKA. Cellular levels of cAMP are increased by activation of the andeylate cyclase, Cyr1p (**Figure 1.10**) through two distinct G-protein systems: the Ras pathway and the Gpr1-Gpa2 pathway (152).

Ras1p and Ras2p are small GTPases that, in their active GTP-bound state, are capable of binding and activating Cyr1p. Though Ras2p is responsible for the majority of Ras function (153), both of their activities are controlled by the guanine nucleotide exchanged factors (GEFs), Cdc25p and Sdc25p, and the GTPase Activating Proteins (GAPs), Ira1p and Ira2p (154).

Gpr1p is a seven-transmembrane receptor believed to be a sugar sensing receptor (155) that, together with its heterotrimeric G-protein α subunit Gpa2p, regulate filamentous growth (156) in a nutrient-responsive manner. Upon glucose binding, Gpr1p activates Gpa2p, which then binds and activates Cyr1p. Gpa2p has also been shown to interact with two PKA inhibitors, Gpb1p and Gpb2p, to alleviate repression of the PKA holoenzyme (157).

Both pathways converged on and activate Cyr1p, which stimulates cAMP production. Intracellular cAMP then binds to the regulatory subunit of the PKA holoenzyme, Bcy1p, freeing and activating the catalytic subunits, Tpk1p, Tpk2p, and Tpk3p. Interestingly, though these proteins share nearly 75% sequence similarity (158), each protein induces different target genes (159), and deletion of each gene produce disparate phenotypes: *tpk1*Δ has no effect, *tpk2*Δ abolishes filamentous growth, and *tpk3Δ* leads to hyperfilamentous growth. While the downstream targets of Tpk3p are still unclear, the substrates of Tpk2p include the filamentous growth transcription factor Flo8p. Similarly, Tpk1p has been shown to regulate the dual-specificity tyrosine-regulated kinase Yak1p, which positively regulates filamentous growth through the transcription factors Sok2 and Phd1 (160).

1.4.1.1.3. The Snf1p kinase pathway

Snf1 protein kinase belongs to the highly conserved Snf1/AMP-activated protein kinase (AMPK) family. Though first identified by its requirement for growth on carbon sources less

preferred than glucose such as sucrose, galactose, or ethanol, (161), recent studies have demonstrated a role for Snf1p directly in glucose signaling (162) and in numerous nutrient-responsive, developmental processes including meiosis (163), aging (164), autophagy (165), and filamentous growth (131, 166). Snf1p is activated by a poorly understood signal beginning with glucose limitation or other environmental stresses (167), and its catalytic activity is controlled by several methods including three upstream kinases, Sak1p, Elm1p, and Tos3p, the Reg1-Glc7 protein phosphatase 1, and by auto-inhibition (168). Snf1p, itself, controls the transcriptional changes associated with glucose derepression through its activation of the transcriptional activators Cat8p and Sip4p, and its deactivation of the transcriptional repressor Mig1p to regulate glucose-repressed genes (**Figure 1.11**). Notably, glucose limitation appears to induce filamentous growth through Snf1p in a pathway independent of Gpr1p by regulating the *FLO11* repressors Nrg1p and Nrg2p (130, 169). Similarly, nitrogen deprivation has been shown to stimulate the filamentous growth response via Snf1p (170). These observations are significant because they establish Snf1p as a consistent connection between the many nutritional states required for filamentous growth. (135)

1.4.1.1.4. The Target-Of-Rapamycin (TOR) pathway

The Target-Of-Rapamycin (TOR) is a conserved Serine/Threonine protein kinase. The TOR pathway has been implicated in the integration and regulation of cellular responses to nutrients (reviewed in (171–173) (**Figure 1.12**). In yeast, there are two distinct TOR kinases, Tor1p and Tor2p, which work in conjunction with the mediator protein Tap42p. In response to particular nutritional signals, TOR kinases phosphorylate Tap42p, which is then able to bind and inhibit type 2A phosphatases such as Sit4p to control cytoplasmic translation initiation and other downstream functions of TOR (154), including cytoplasmic protein synthesis, protein degradation, and cell cycle progression (174) as well as inhibiting the activity of transcription factors involved in nitrogen catabolite-repression (NCR) (Gat1p, Gln3p) (175), retrograde (RTG) response (Rtg1p, Rtg3p) (176), and stress-response (Msn2p, Msn4p) (177, 178), while promoting the function of transcriptional regulators involved in ribosome biogenesis (Fhl1p, Spf1p) (179). The protein kinase Sch9p is also directly regulated by TOR in a stress-dependent manner. Sch9p has been connected to the cellular response to nutrient deprivation, replicative lifespan, ribosome

biogenesis, and osmotic stress (180) suggesting its role as a key channel for TOR signal transduction (149).

The TOR signaling cascade also plays a conserved role in regulating filamentous differentiation in response to nutrients, particularly nitrogen availability, (181) and appears to be independent of RAS/PKA and MAPK pathways. The ammonium permease, Mep2p, and the downstream NCRs Gln3p, Ure2p, and Npr1p are required for filamentous growth (182). Similarly, the TOR pathway was found to regulate transcription factor Gcn4p, a positive regulator of genes expressed during amino acid starvation that also mediates *FLO11* expression (183). Interestingly, recent data indicates the existence of two, distinct nitrogen-responsive pathways suggesting that the regulation of NCR and RTG genes is not solely TOR dependent (184, 185). The nature of this pathway and its components is unclear, but the Npr2p/Npr3p complex, a nitrogen permease regulator and required for growth in low nitrogen ((186) and Unpublished data), could be members of this pathway (135).

1.4.1.1.5. Other components that regulate filamentous growth

Noticeably, *S. cerevisiae* exploits multiple signaling mechanisms to response to various environmental cues (**Figure 1.13**). Nitrogen limitation induces a dimorphic switch where yeast cells transition into filamentous pseudohyphae, presumably to scavenge for nutrients. This filamentous transition is regulated by a complex system of signaling pathways that include MAPK, cAMP-PKA, SNF1, and TOR, which all appear to converge, in some manner, on *FLO11* as described above and reviewed in (187) (**Figure 1.14**). In addition, recent studies have identified a transcription factor cascade comprised of Sok2, Phd1, Ash1, and Swi5 that controls cell–cell adhesion and separation (188) (reviewed in (32) as well as numerous cis-acting regulators of *FLO11* that do not readily fit in the above pathways (189). Furthermore, a systematic analysis of the yeast kinome identified six cytoplasmic proteins (Bcy1p, Fus3p, Ksp1p, Kss1p, Sks1p, and Tpk2p) that translocated to the nucleus under filamentous conditions and were required for filamentous growth (190). Interestingly, the Sks1p kinase is poorly characterized and only known to play a role in the cellular response to extracellular glucose (191, 192), leaving its involvement in the complex phenotypic response that is filamentous growth to be determined.

In Chapter 2, I present a study that leverages a novel bioinformatics tool for identifying biological meaningful relationships in high-throughput data to discover novel regulators of yeast stress response and filamentous growth (108). Specifically, the Topology Enrichment Analysis framework (TEAK) identified two, previously unreported genes necessary for the yeast nitrogen stress response as well as a gene involved in lipid metabolism found to be required for filamentous growth.

Chapter 3 of this text presents a study of the Sks1p kinase and its involvement in filamentous growth. Here, a large-scale proteomics approach was used to investigate the contributions of kinase signaling to the regulation of filamentous growth. We identified 903 phosphopeptides 62 of which exhibited a two-fold decrease in phosphorylation and 52 of which demonstrated a two-fold increase in phosphorylation in a Sks1p kinase dead strain. The data was used to further characterize Sks1p in order to identify its mechanism of filamentous control. Fourteen putative Sks1p targets were screened for involvement in filamentous growth, and six proteins were found to be involved for pseudohyphal differentiation. In addition, two identified phosphorylated residues were found to be functionally required for filamentous growth.

1.4.2. Filamentous growth in *C. albicans*

As previously described, invasive fungal infections are a serious health concern, particularly in individuals with weakened immune systems. Immunocompromised individuals are very susceptible to nosocomial infections, those acquired while in the hospital, and *Candida albicans*, a human commensal, is the predominant human nosocomial fungal pathogen (193). *C. albicans* is responsible for a spectrum of disease states ranging from superficial mucosal infections to life-threatening systemic disease (194–196) that have an estimated mortality rate in excess of 30% (197).

First identified in the early 19th century by physicians studying the cause of oral thrush in infants (**Figure 1.15**), little of its biology was elucidated until the latter half of the 20th century (for an excellent historical review of *C. albicans* see (198)). The lag in scientific advancement is partially due to unique genetic characteristics of this organism. Specifically, *Candida* does not exist as a stable haploid, making genetic manipulation more complicated than in *S. cerevisiae*. (Note: The idea of that *Candida albicans* is an "obligate diploid", though longstanding, was recently challenged by the discovery of mating-competent haploids (199).) Likewise, the lower

frequency of homologous recombination (200), its non-standard genetic code (CUG codon codes as Leucine instead of Serine (201)), lack of naturally occurring replicating plasmids, and a natural resistant to common antibiotics (202) have limited classical and targeted genetic approaches (203). The past couple of decades, however, have seen a dramatic increase in the array of experimental tools and improved protocols for experimental analysis of *Candida albicans* (reviewed in (204)), which has greatly increased our understanding of its biology.

C. albicans is polymorphic in that it is capable of growing in at least three different morphologies: budding yeast, pseudohyphae, or true, septate hyphae (**Figure 1.16**). (*S. cerevisiae* cannot form true hyphae). Each of these forms is believed to be a response to its environment. Notably, the ability to switch between yeast, hyphal and pseudohyphal morphologies is regularly considered necessary for virulence (205), as mutants incapable of switching to hyphal form have been shown to be hypovirulent (114, 206, 207). In the lab, morphological switching from yeast to filamentous forms can be induced by a variety of conditions. These conditions include growth at elevated temperature (37°C) coupled with neutral pH, the presence of serum, certain human hormones, Spider medium, or Lee's medium (208–213). The signaling pathways that transmit these environmental signals result in the activation of a complex regulatory network central to *C. albicans* filamentous growth and virulence. Interestingly, at least three of these pathways are highly conserved in *Saccharomyces cerevisiae*: the cAMP-dependent PKA pathway, the MAPK pathway, and the Regulation of Ace2 and Morphogenesis (RAM) pathway. The cAMP and MAPK pathways in budding yeast are directly connected to pseudohyphal growth, while the RAM pathway appears to be exhibit some modulation of pseudohyphal development in a manner seemingly independent of nutritional status (214, 215). The conservation and signaling dynamics of the cAMP-PKA, MAPK, and RAM pathways among filamentous fungi was recently reviewed in (33, 216, 217), respectively.

Figure 1.1. Early drawing of yeast. Image created by van Leeuwenhoek in his letter to Thomas Gale written June 14, 1680.

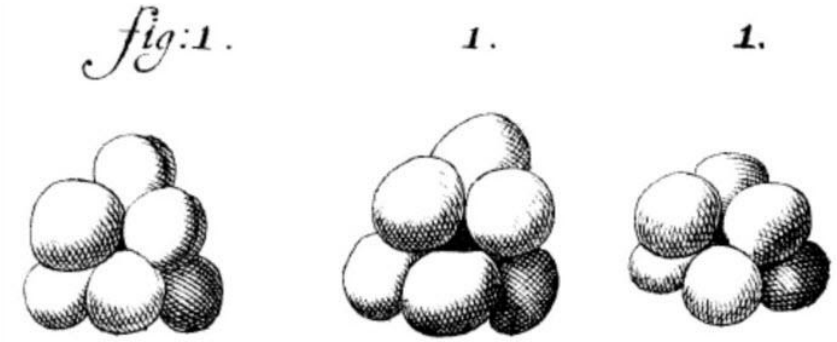


Figure 1.2. Uses of yeast in biotechnology. Modified from (8)

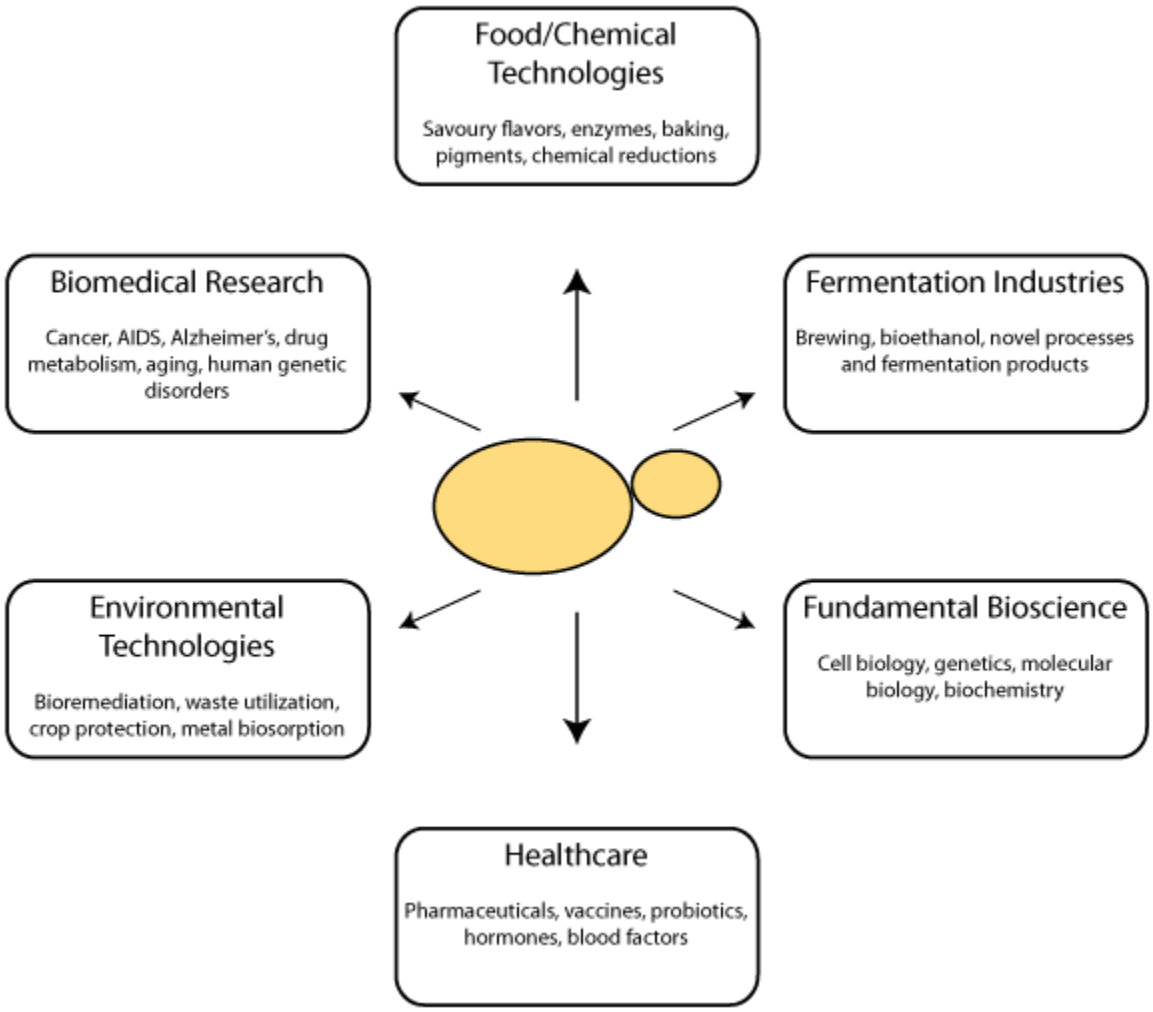


Figure 1.3. Gene expression analysis using a DNA microarray. In this illustration, mRNA samples from vegetative and sporulating yeast cells are compared. The total pool of messenger RNA from each cell population is used to prepare fluorescently labeled cDNA by reverse transcription in the presence of fluorescently labelled nucleotide precursors. To allow direct comparison of the abundance of each gene in the two samples, the two samples are labeled with different fluors. In this example, a red fluor for the mRNA from sporulating yeast and a green fluor for the mRNA from the vegetative yeast cells are used. The two fluorescently labelled cDNAs are then mixed and hybridized with a DNA microarray in which each yeast gene is represented as a distinct spot of DNA. Irrespective of their fluorescent labels, the cDNA sequences representing each individual transcript hybridize specifically with the corresponding gene sequence in the array. Thus, the relative abundance in sporulating as compared with vegetative yeast cells of the transcripts from each gene is reflected by the ratio of 'red' to 'green' fluorescence measured at the array element representing that gene. For example, the greater relative abundance of the TEP1mRNA in the sporulating cells results in a high ratio of red-labelled to green-labelled copies of the corresponding cDNA, and an equivalent ratio of red to green signal hybridized at the array element composed of DNA from TEP1.(218)

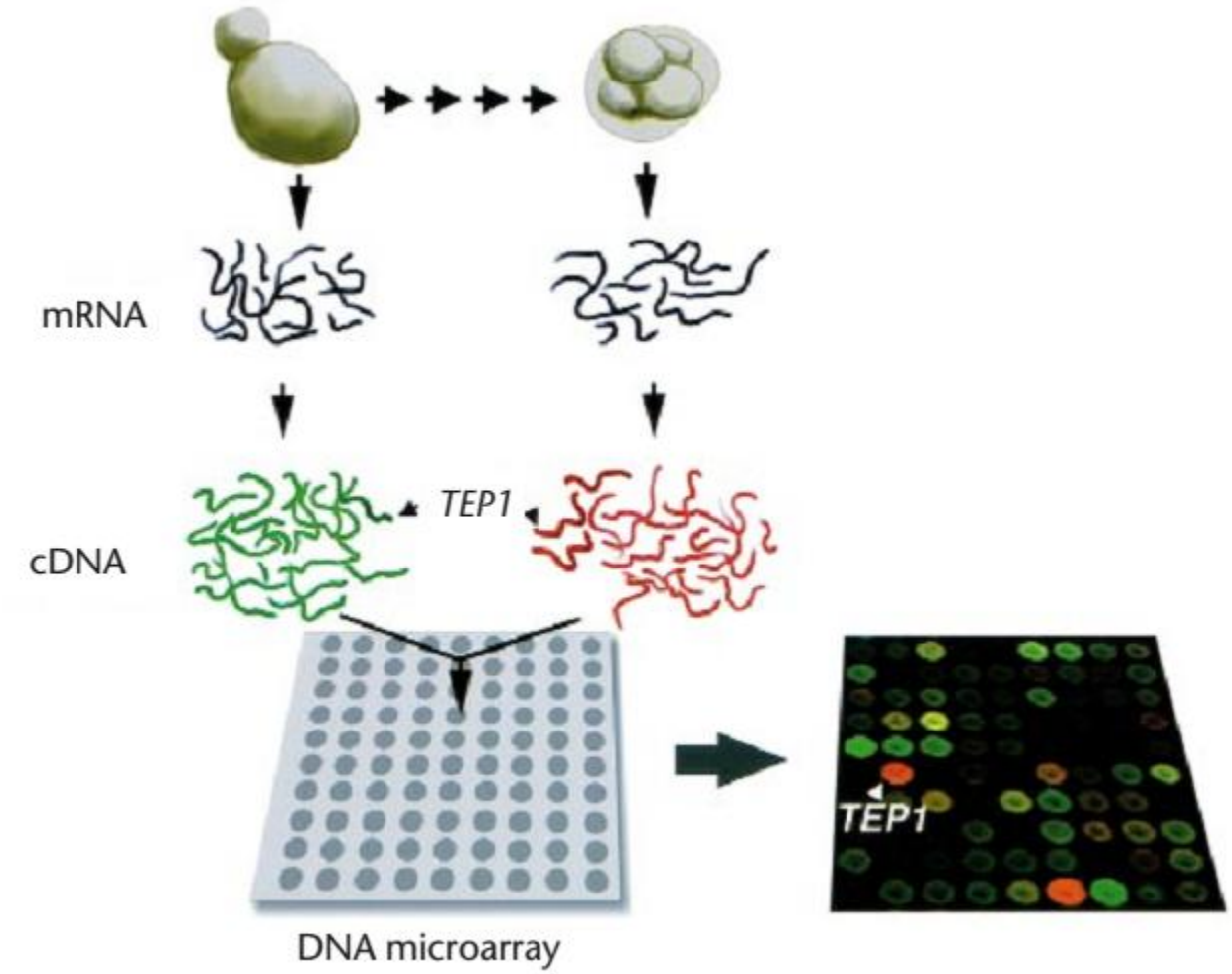


Figure 1.4. A typical RNA-seq experiment. Briefly, long RNAs are first converted into a library of cDNA fragments through either RNA fragmentation or DNA fragmentation (see main text). Sequencing adaptors (blue) are subsequently added to each cDNA fragment and a short sequence is obtained from each cDNA using high-throughput sequencing technology. The resulting sequence reads are aligned with the reference genome or transcriptome, and classified as three types: exonic reads, junction reads and poly(A) end-reads. These three types are used to generate a base-resolution expression profile for each gene, as illustrated at the bottom; a yeast ORF with one intron is shown.(68)

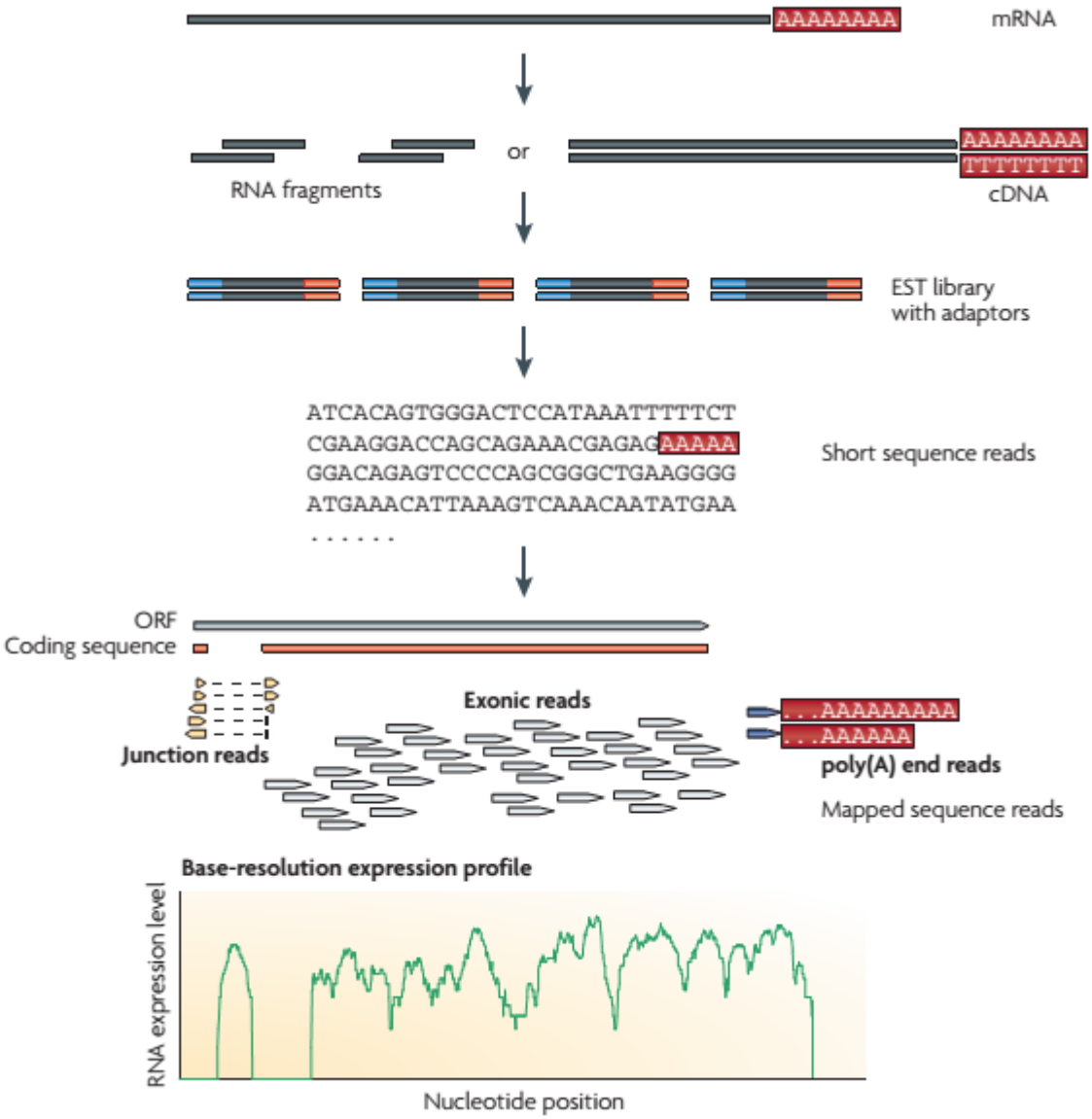


Figure 1.5. The first sketch of the two-hybrid assay. Image as seen in a grant application submitted at the end of 1987 to the Procter and Gamble Company. The native yeast Gal4 protein is shown as having DNA-binding (GAL4D) and activation (GAL4A) domains, with the DNA-binding domain recognizing a site on DNA known as the Upstream Activation Sequence for the GAL genes (UASG). Proteins P and Q form hybrids with the DNA binding and activation domains, respectively, and reconstitute transcriptional activity, leading to expression of the GAL1 gene.(74)

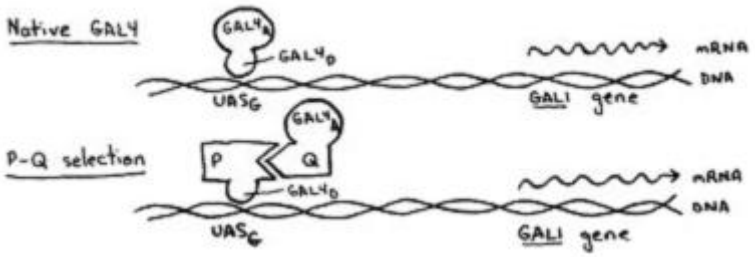


Figure 1.6. Generalized mass-spectrometry-based proteomics workflow. Extracted proteins are first digested into peptides, usually by trypsin. Enzymatic digestion of a full proteome generates hundreds of thousands of peptides, thus, the first step in the proteomics workflow is most often reducing the sample complexity via sample prefractionation or enrichment. These reduced samples are then introduced to the LC system for an additional separation step. After ionization, peptide precursor ions are introduced into the mass spectrometer, which records their mass-to-charge (m/z) ratio with high accuracy. For identification, single precursors are selected (on the basis of observed intensity) and subjected to a tandem MS (MS/MS) event to generate characteristic fragment ions for the selected precursor. The combination of precursor m/z and its fragment ions is then matched to known peptide sequences from large protein databases. Finally, data are quantified. These protein abundances are then interpreted and visualized in the context of the biological system under study. HILIC, hypdrophilic interaction liquid chromatography. (82)

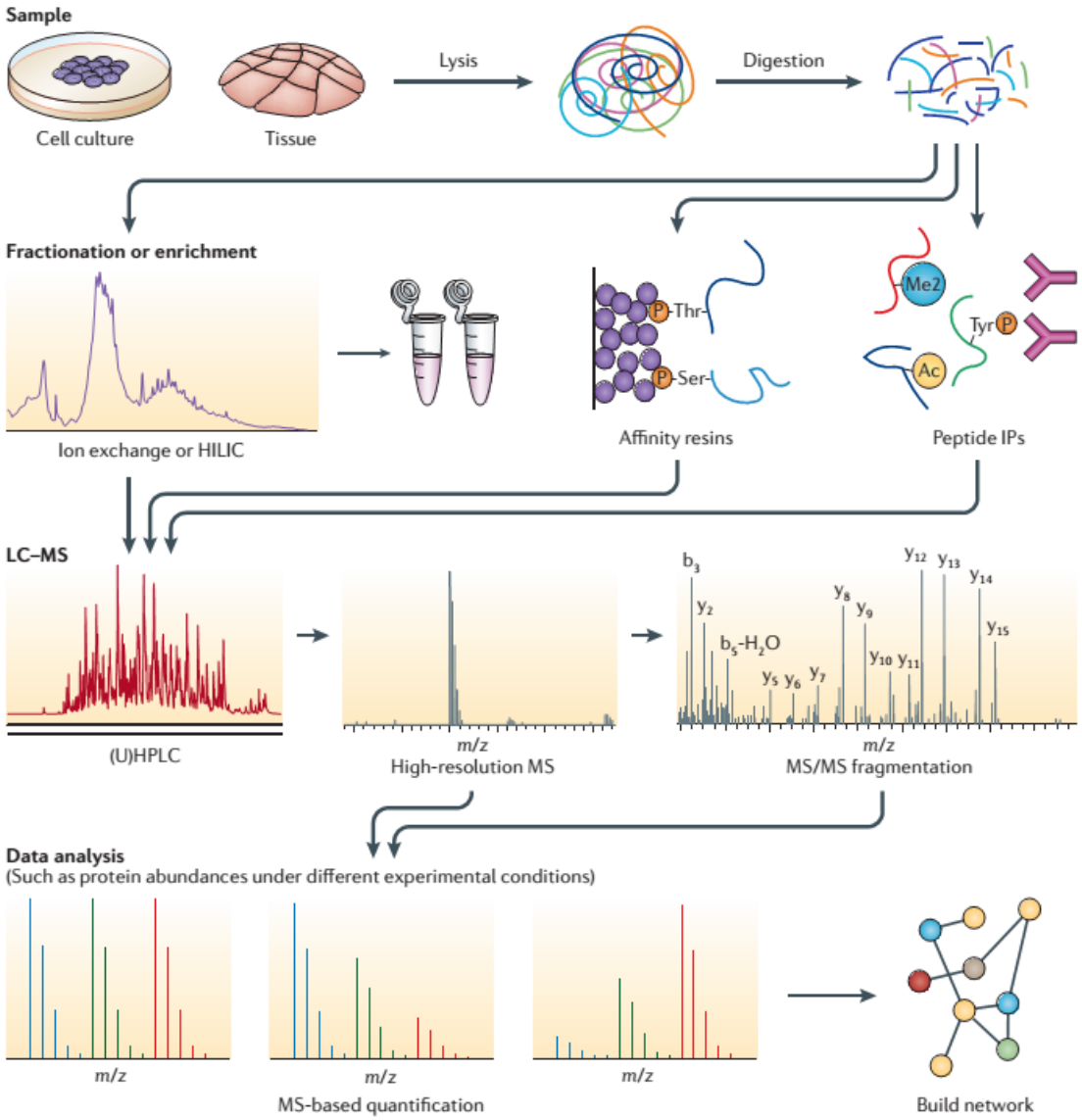


Figure 1.7. Overview of SILAC experimentation in yeast.

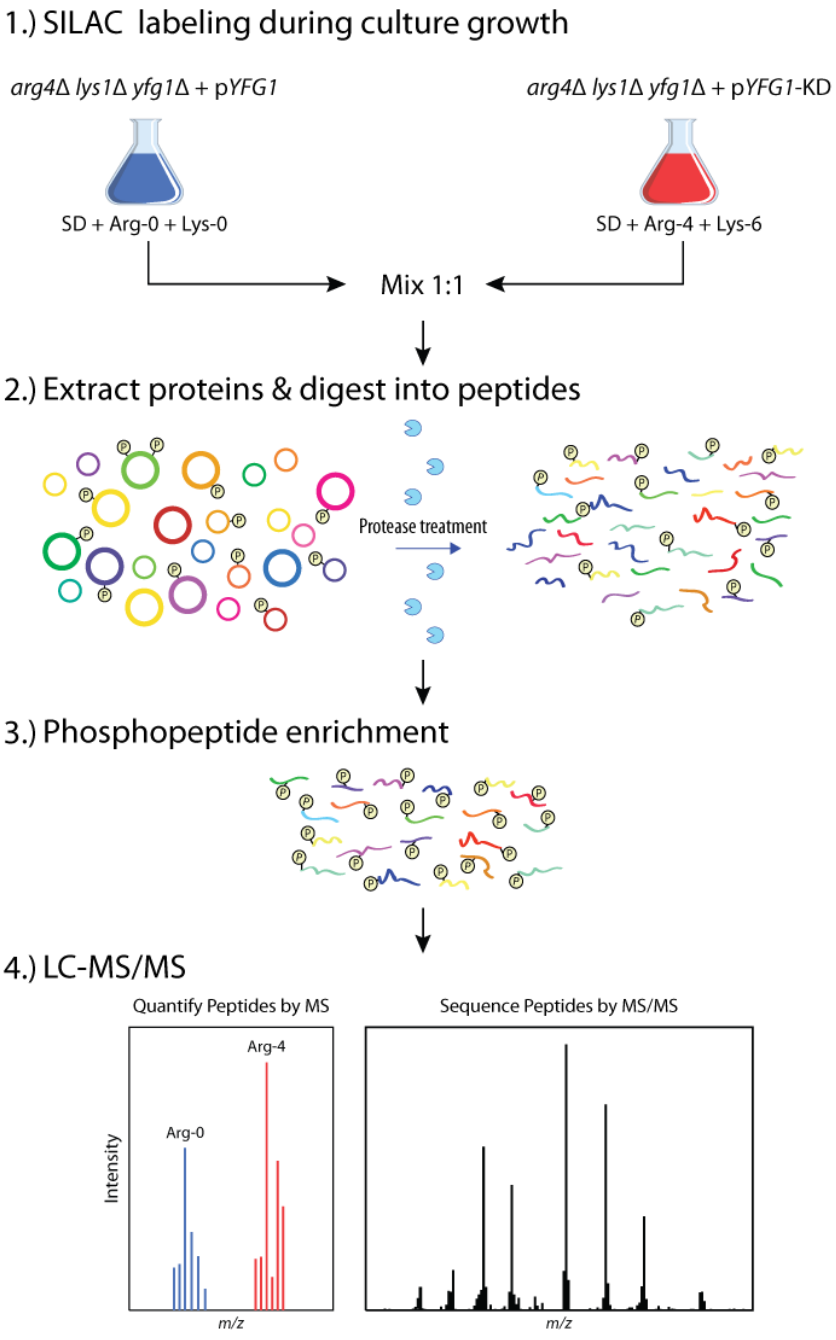


Figure 1.8. Diploid cells of the yeast *Saccharomyces cerevisiae* undergo pseudohyphal differentiation in response to nitrogen limitation. In response to nitrogen-limiting conditions, diploid cells of *S. cerevisiae* change their growth pattern: the cells elongate and switch from bipolar to unipolar budding, the mother and daughter cells remain physically attached, and the cells invade the growth substrate. As a result, filamentous pseudohyphal colonies are formed.(188)

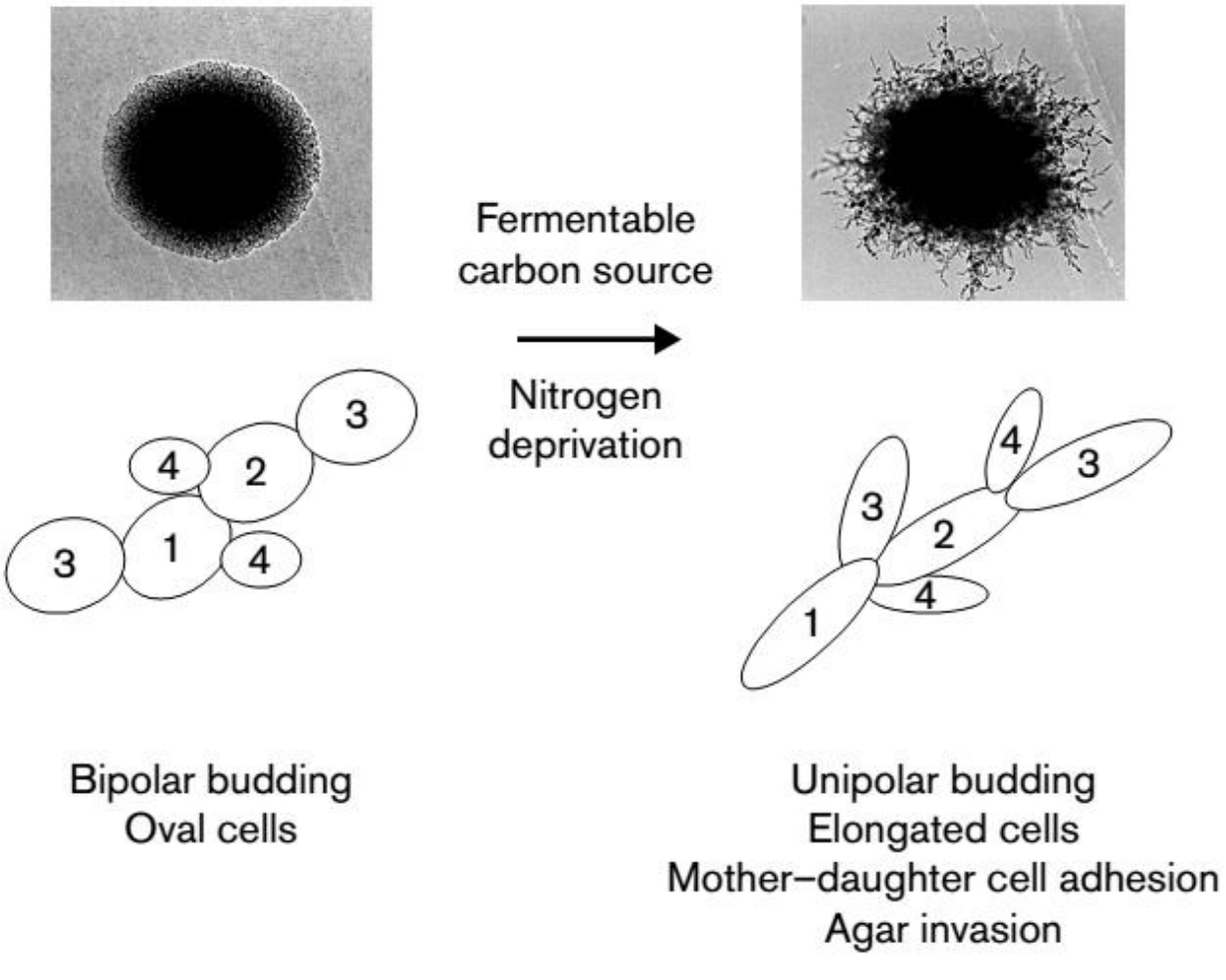


Figure 1.9. Three MAPK pathways in yeast share common components and also contain pathway-specific factors. Three MAPK pathways are shown. Colored proteins represent pathway-specific factors; proteins shown in black function in multiple pathways. Scaffold-mediated interactions are shown by colored, dashed lines. Not all protein interactions are shown. Hot1 is one of a number of transcription factors for the HOG pathway. The red question mark indicates that how nutritional signals feed into filamentous growth pathway regulation is not well understood. (34)

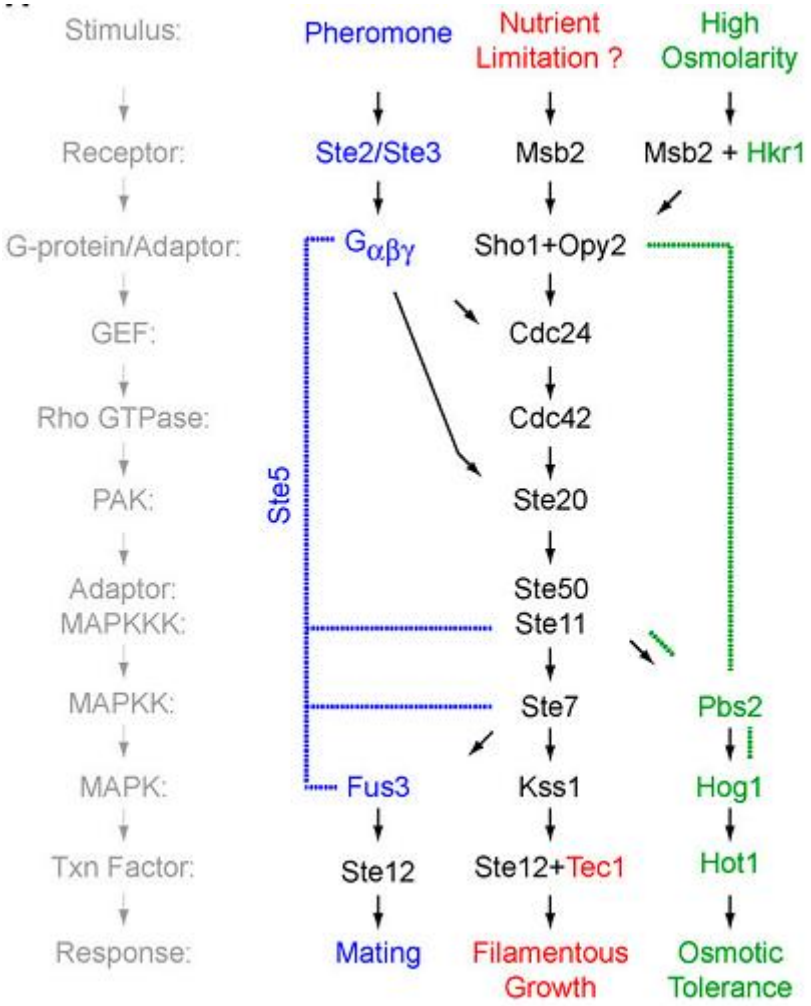


Figure 1.10. G-protein based glucose signaling. Glucose signaling mediated by the small G-proteins Ras and Gpa2 funnels through protein kinase A (PKA) to induce ribosome biogenesis and suppress the general stress response controlled by Msn2/Msn4 and Rim15. The kinases Sch9 and Yak1 function in parallel to PKA, the former reinforcing the PKA response and the latter antagonizing it. Dashed lines represent regulatory interactions, which may not be direct and in some cases are only surmised. (149)

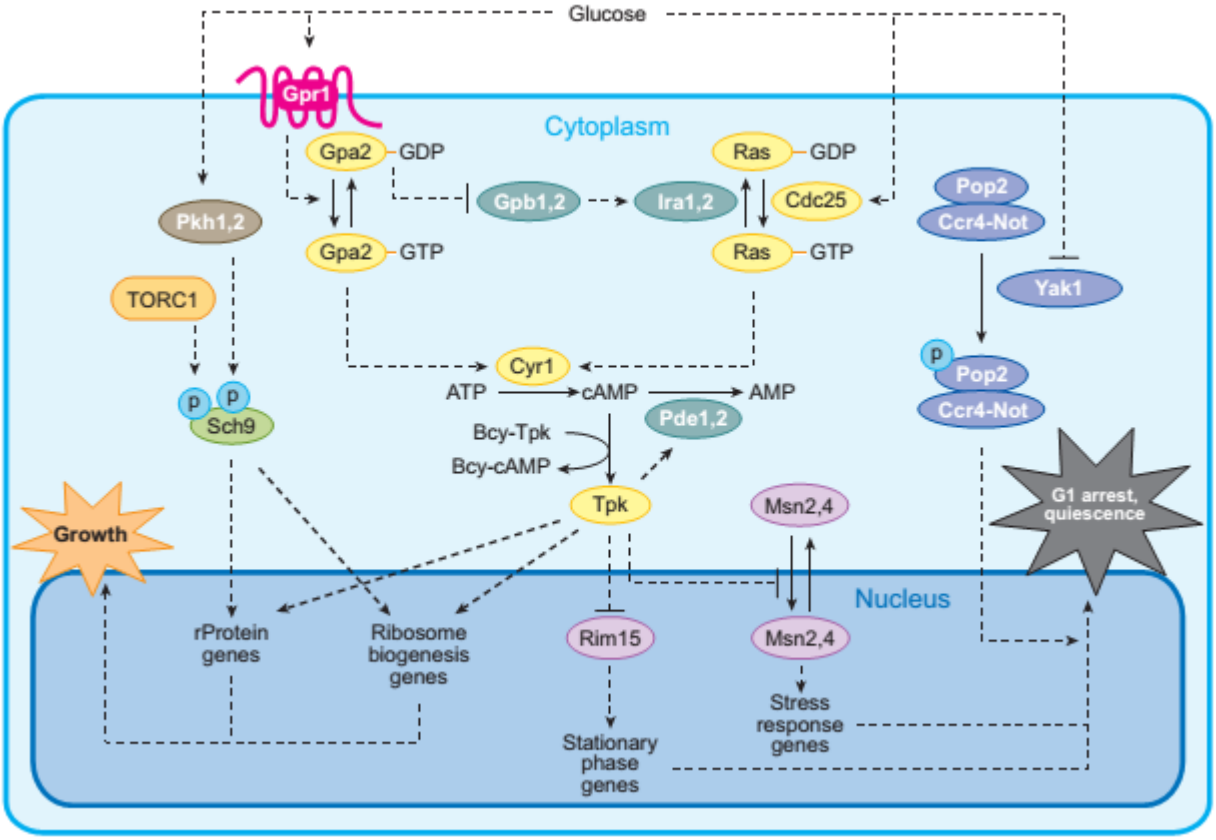


Figure 1.11. The main glucose repression pathway in *S. cerevisiae*. In the presence of high levels of glucose, the Snf1 kinase complex is inactive due to an auto-inhibitory interaction between the catalytic domain (CD) and the regulatory domain (RD) of Snf1. Activation of Snf1 upon glucose exhaustion requires the phosphorylation of Thr210 in the activation loop of the catalytic domain of Snf1 and the binding of Snf4 to the regulatory domain of Snf1, which is necessary to lift Snf1 auto-inhibition. The phosphorylation status of the threonine residue is controlled by the upstream kinases Sak1, Elm1 and Tos3 and the Glc7–Reg1 phosphatase complex. Activated Snf1 phosphorylates Mig1, thereby stimulating the translocation of the repressor to the cytoplasm, which relieves several gene families of glucose repression. The glucose signal that controls Snf1 activity is possibly transduced via Hxk2 to Glc7–Reg1 and via a sensing of the AMP/ATP ratio by Snf4.Arrows and bars represent positive and negative interactions, respectively. Dashed lines are putative or indirect interactions. (173)

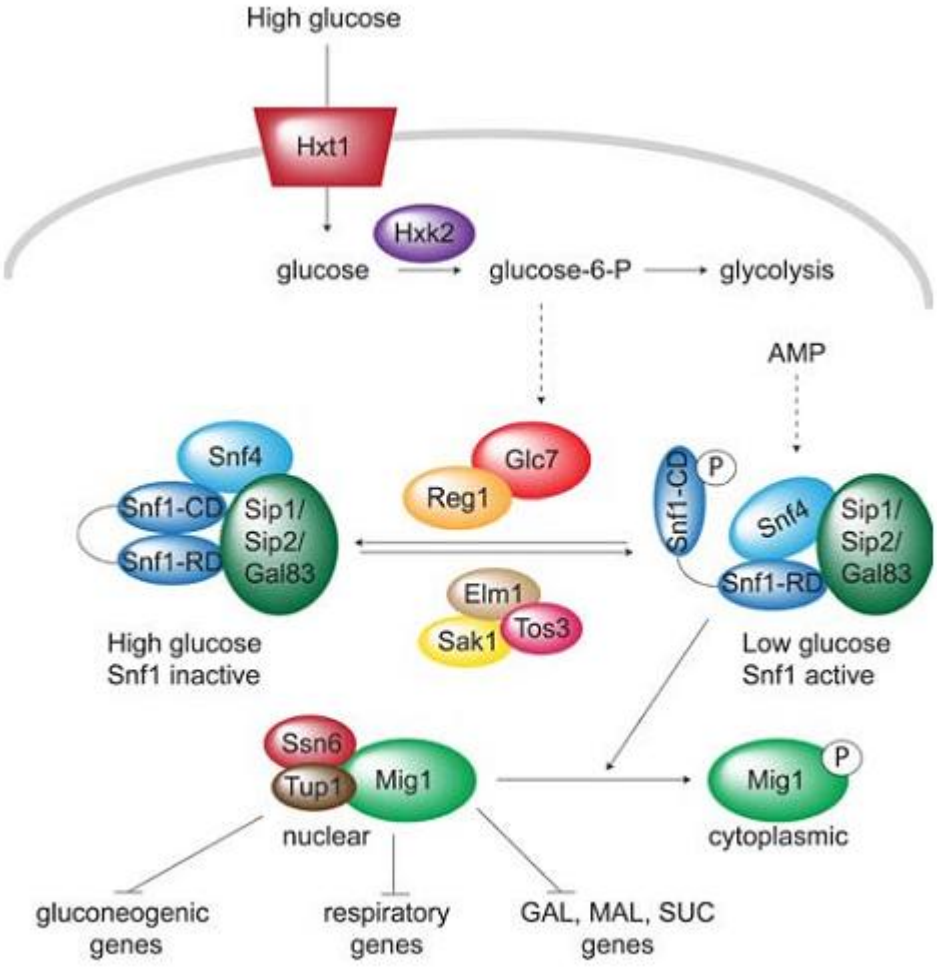


Figure 1.12. TORC1 and nitrogen regulation. Two pathways, one mediated by TORC1 and a second less well-defined nitrogen catabolite repression pathway, adjust growth as well as expression of genes required for use of alternate nitrogen sources in response to the quality and quantity of available nitrogen sources through regulation of transcriptional activators (blue icons) and repressors (red icons). TORC1 likely responds to intracellular amino acid levels sensed through the Ego complex and regulates growth primarily through Sch9, regulates stress, and alternative nitrogen source through protein phosphatase 2A and regulates permease sorting through Npr1. Npr2/3 lie upstream of NCR gene expression, but whether they regulate TORC1 or the ill-defined NCR pathway is not clear. (135)

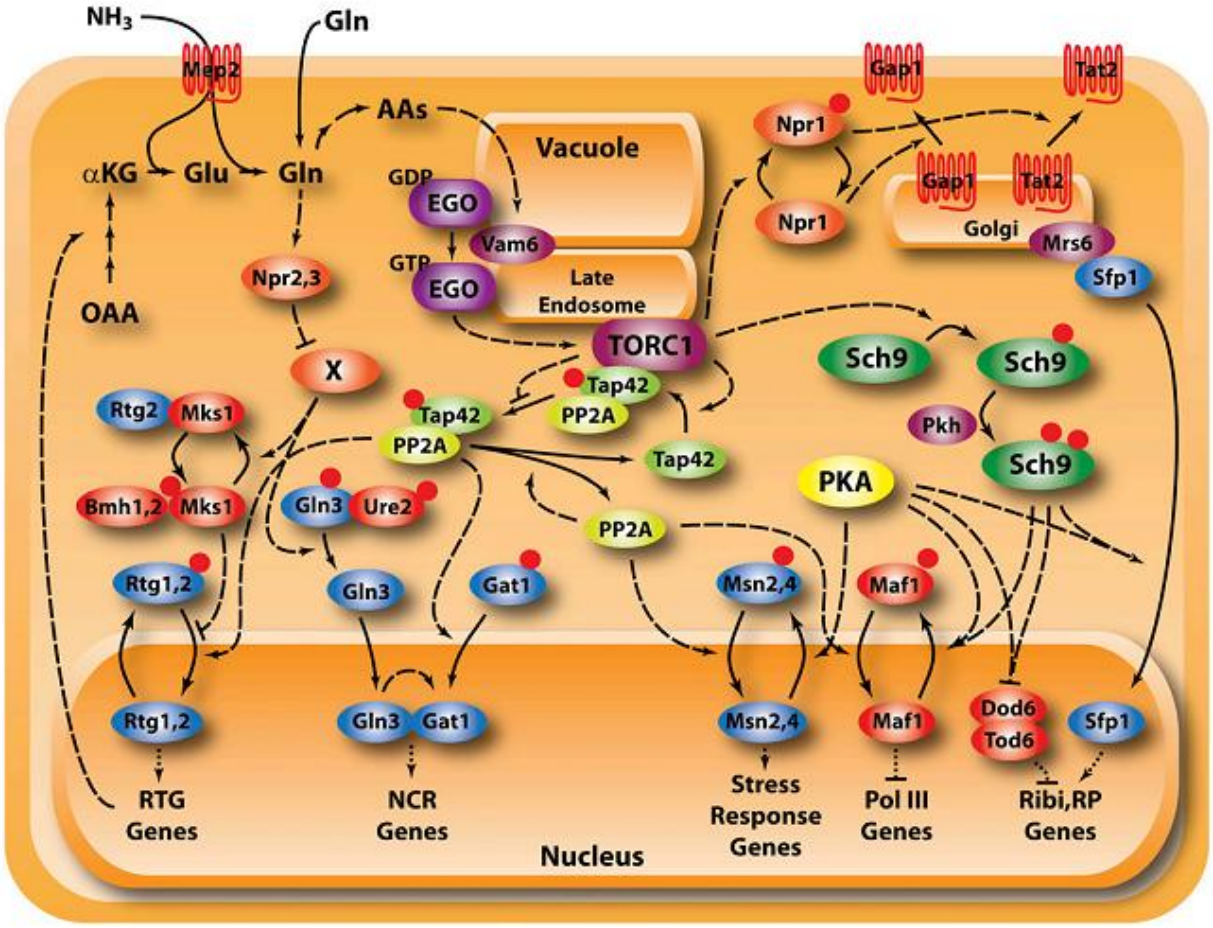


Figure 1.13. A simplified view of the glucose sensing mechanisms in *S. cerevisiae.* A schematic view of the three glucose sensing pathways in yeast. The major interconnections with the TOR pathway (only partially shown) are indicated.

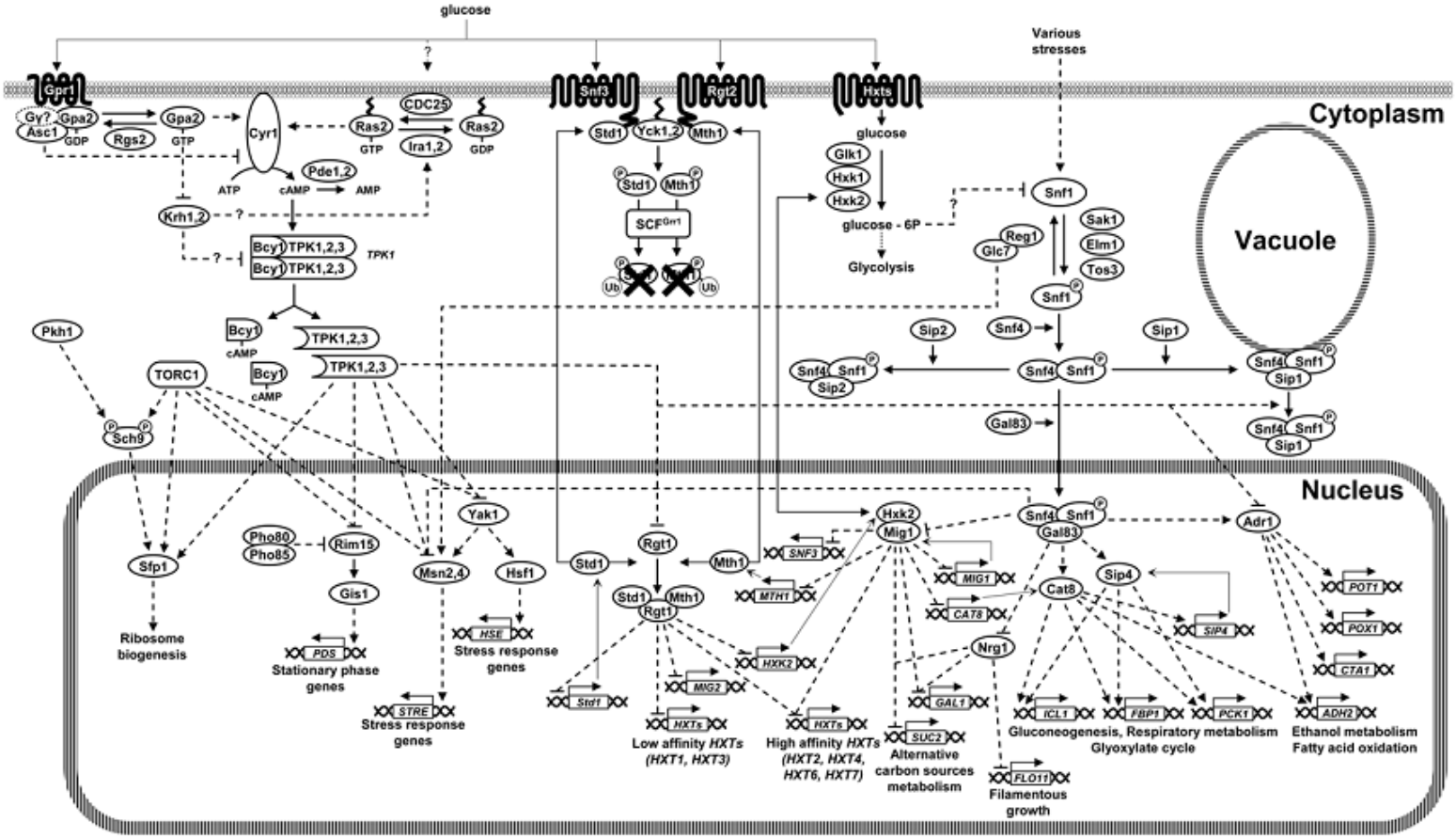


Figure 1.14. Integrative network of cAMP-PKA, MAPK and TOR pathways involved in the regulation of filamentous growth in *Saccharomyces cerevisiae*. Mep2 functions upstream of cAMP and MAPK pathways as an ammonium sensor. Kelch repeat protein Gpb1/2 antagonizes Gpa2 and PKA of cAMP pathway and stabilizes Ira1, which inactivates Ras2 of MAPK pathway. In cAMP-PKA pathway, Ras2 and Gpa2 activate adenylate cyclase, Cyr1 to synthesis cAMP, which binds to PKA and relieves the inhibition of catalytic subunits Tpk1, Tpk2 and Tpk3. Tpk2 activates the transcriptional activator Flo8 involved in the regulation of FLO11. In the MAPK pathway, Ras2 and Sho1 activates the Cdc42-Ste20 complex, which in turn activates the MAPK cascade Ste11, Ste7 and Kss1 to control the transcriptional activator Ste12-Tec1. Nitrogen starvation or rapamycin treatment is shown to inactivate the TOR pathway. Tor kinase phosphorylates Tap42, which complexes with phosphatase Sit4 and Pph21/22. Further, Sit4 and Pph21/22 controls Gln3 mediated NCR genes and Msn2/4 mediated STRE genes, respectively. Tor-activated Tap42 also participate in the global translational initiation. TOR pathway exerts translational control over G1 cyclin Cln3, which in turn controls the synthesis of other G1 cyclin Cln1/2 through transcriptional activator SBF. Cln1/2 is destabilized by Grr1 and is involved in the transcriptional activation of FLO11.(187)

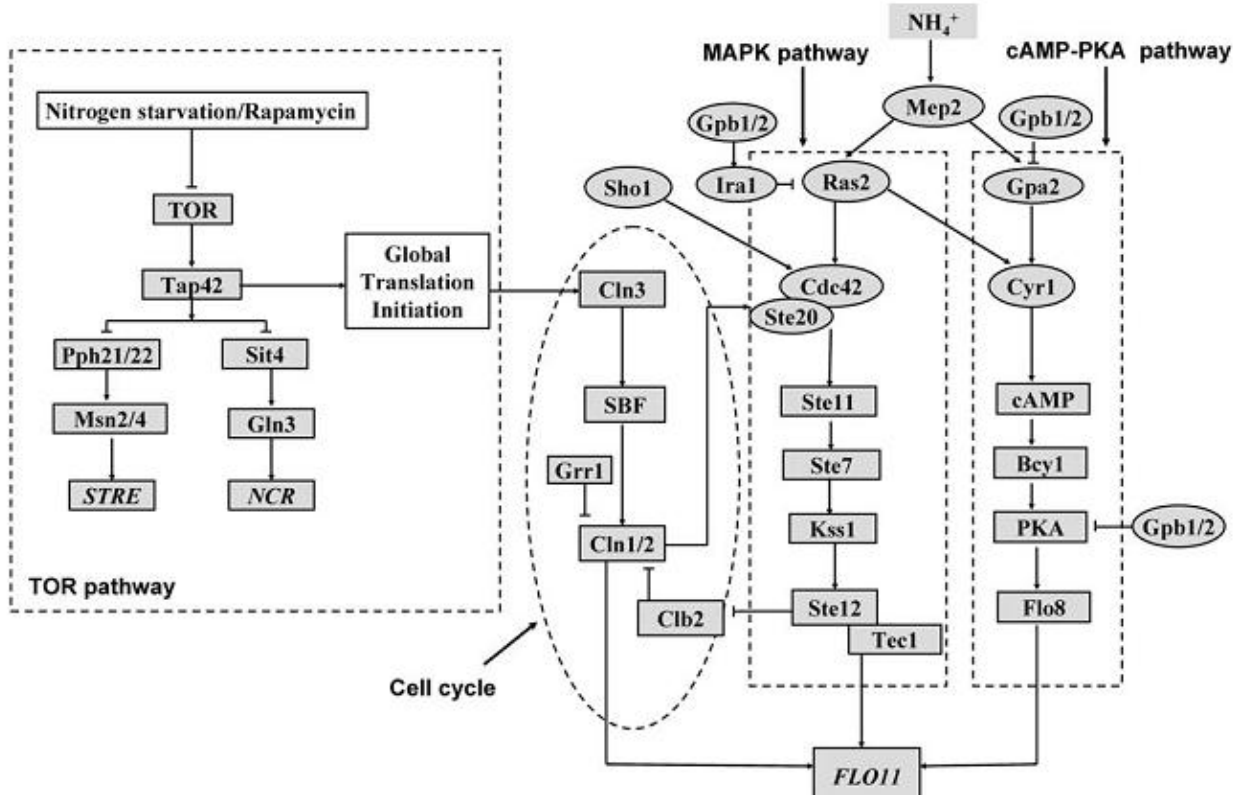


Figure 1.15. The first printed illustrations of *Candida (Oidium) albicans*: the thrush fungus (*champignon du muguet*). Plate I from Charles Robin's 1853 publication. Illustrates epithelial fragments affected by thrush, with round cells of *C .albicans* and its filaments bearing characteristic ball-like clusters of blastoconidia (Fig 3c and 5f) (198).

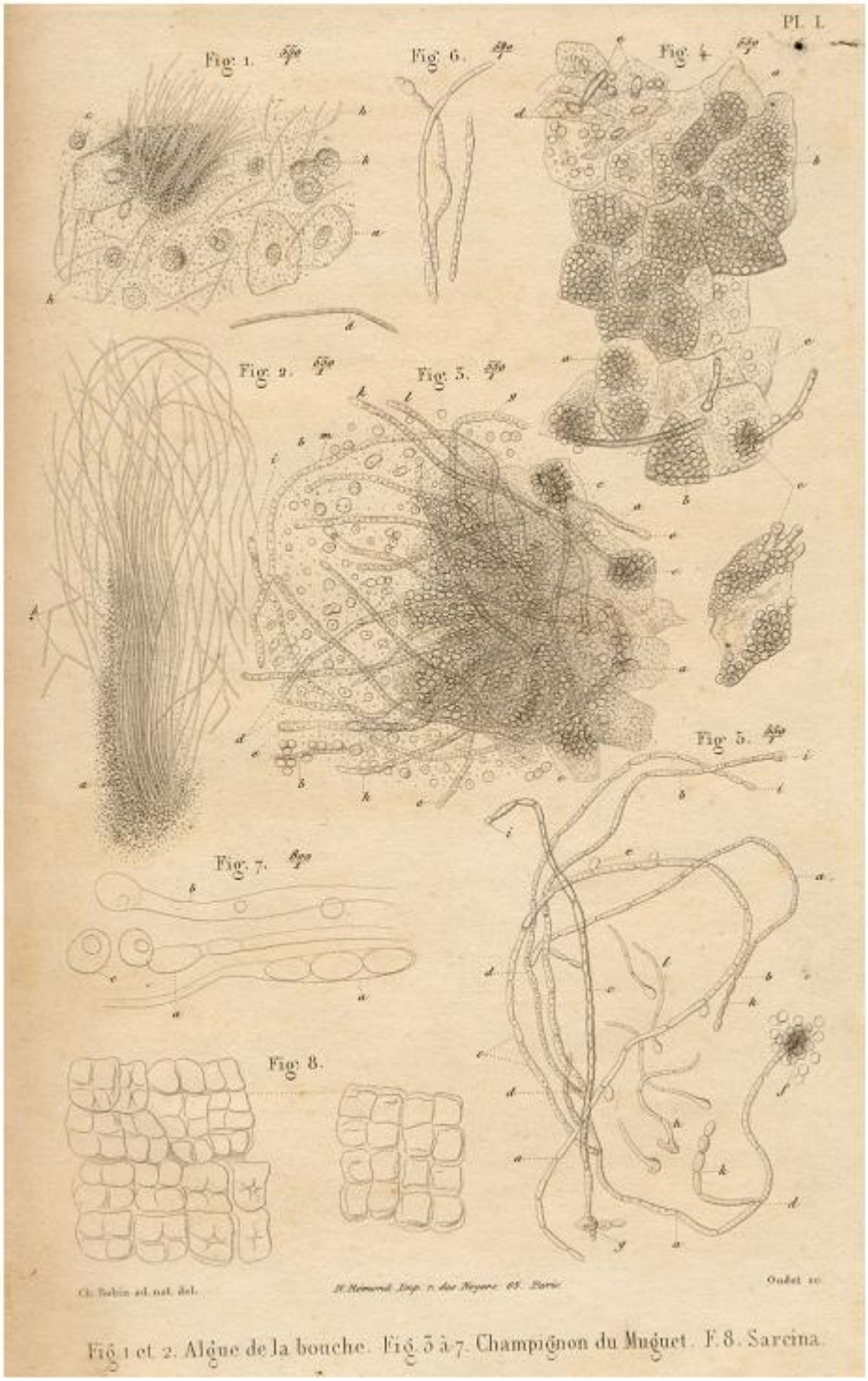
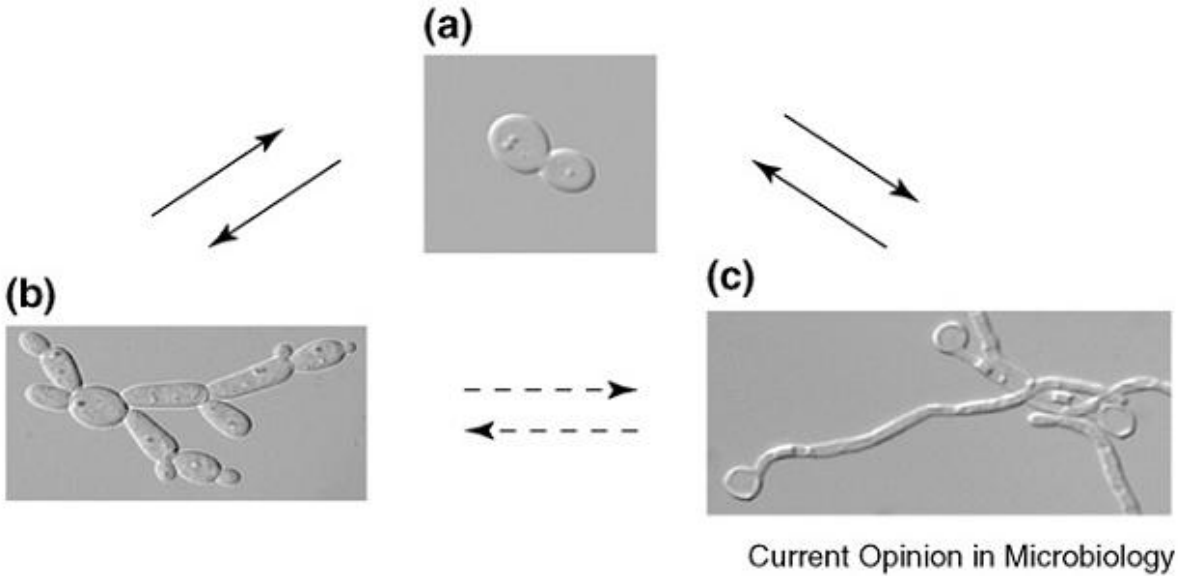


Figure 1.16. Differential morphology of C.albicans cells. (a)Yeast cells can form both (b) pseudohyphae and (c) true hyphae. Switching between the pseudohyphal and hyphal morphologies is less frequent.(219)



References

1. Chambers PJ, Pretorius IS (2010) Fermenting knowledge: the history of winemaking, science and yeast research. *EMBO reports* 11:914–20.

2. Feldmann H (2005) in *Yeast Molecular Biology: A Short Compendium on Basic Features and Novel Aspects* (Adolf-Butenandt-Institute, Munich).

3. McGovern PE (2010) *Uncorking the Past: The Quest for Wine, Beer, and Other Alcoholic Beverages* (University of California Press, Berkeley).

4. Barnett JA (1998) A history of research on yeasts. 1: Work by chemists and biologists 1789-1850. *Yeast (Chichester, England)* 14:1439–51.

5. Barnett JA (2000) A history of research on yeasts 2: Louis Pasteur and his contemporaries, 1850-1880. *Yeast (Chichester, England)* 16:755–71.

6. Fruton JS (1972) *Molecules and Life* (Wiley-Interscience, New York).

7. Barnett JA (2003) Beginnings of microbiology and biochemistry: the contribution of yeast research. *Microbiology (Reading, England)* 149:557–67.

8. Walker GM (2009) in *Encyclopedia of Microbiology*, ed Schaechter M (Academic Press, Oxford), pp 478–491.. 3rd Ed.

9. Kim I-K, Roldão A, Siewers V, Nielsen J (2012) A systems-level approach for metabolic engineering of yeast cell factories. *FEMS yeast research* 12:228–48.

10. Nevoigt E (2008) Progress in metabolic engineering of Saccharomyces cerevisiae. *Microbiology and molecular biology reviews : MMBR* 72:379–412.

11. Hilleman MR (1987) Yeast recombinant hepatitis B vaccine. *Infection* 15:3–7.

12. Hong K-K, Nielsen J (2012) Metabolic engineering of Saccharomyces cerevisiae: a key cell factory platform for future biorefineries. *Cellular and molecular life sciences : CMLS* 69:2671–90.

13. Roman H (1981) in *The Molecular and Cellular Biology of the Yeast Saccharomyces*, eds Strathern JN, Jones EW, Broach JR (Cold Spring Harbor Laboratory Press, Cold Spring Harbor).

14. Hinnen A, Hicks JB, Fink GR (1978) Transformation of yeast. *Proceedings of the National Academy of Sciences of the United States of America* 75:1929–33.

15. Beggs JD (1978) Transformation of yeast by a replicating hybrid plasmid. *Nature* 275:104–9.

16. Sherman F (1998) An introduction to the genetics and molecular biology of the yeast Saccharomyces cerevisiae. *Modified from: F. Sherman, Yeast genetics. The Encyclopedia of Molecular Biology and Molecular Medicine*:302–325.

17. Knop M (2006) Evolution of the hemiascomycete yeasts: on life styles and the importance of inbreeding. *BioEssays : news and reviews in molecular, cellular and developmental biology* 28:696–708.

18. Suter B, Auerbach D, Stagljar I (2006) Yeast-based functional genomics and proteomics technologies: the first 15 years and beyond. *BioTechniques* 40:625–44.

19. Goffeau A et al. (1996) Life with 6000 genes. *Science (New York, N.Y.)* 274:546, 563–7.

20. Dolinski K, Botstein D (2005) Changing perspectives in yeast research nearly a decade after the genome sequence. *Genome research* 15:1611–9.

21. Pines M (2001) Discovering What the Genes in a Living Cell Do - Starting with Yeast. *The Genes We Share with Yeast, Flies, Worms, and Mice: New Clues to Human Health and Disease*.

22. Botstein D (1997) GENETICS: Yeast as a Model Organism. *Science* 277:1259–1260.

23. Heinicke S et al. (2007) The Princeton Protein Orthology Database (P-POD): a comparative genomics analysis tool for biologists. *PloS one* 2:e766.

24. Smith MG, Snyder M (2006) Yeast as a model for human disease. *Current protocols in human genetics / editorial board, Jonathan L. Haines ... [et al.]* Chapter 15:Unit 15.6.

25. Rubin GM et al. (2000) Comparative genomics of the eukaryotes. *Science (New York, N.Y.)* 287:2204–15.

26. Li XC, Schimenti JC, Tye BK (2009) Aneuploidy and improved growth are coincident but not causal in a yeast cancer model. *PLoS biology* 7:e1000161.

27. Pereira C et al. (2012) New insights into cancer-related proteins provided by the yeast model. *The FEBS journal* 279:697–712.

28. Pray L (2008) L. H. Hartwell's Yeast: A Model Organism for Studying Somatic Mutations and Cancer. *Nature Education* 1.

29. Tenreiro S, Munder MC, Alberti S, Outeiro TF (2013) Harnessing the power of yeast to unravel the molecular basis of neurodegeneration. *Journal of neurochemistry*.

30. Pimentel C, Batista-Nascimento L, Rodrigues-Pousada C, Menezes RA (2012) Oxidative stress in Alzheimer's and Parkinson's diseases: insights from the yeast Saccharomyces cerevisiae. *Oxidative medicine and cellular longevity* 2012:132146.

31. Truckses DM (2004) Jekyll and Hyde in the Microbial World. *Science* 306:1509–1511.

32. Lengeler KB et al. (2000) Signal transduction cascades regulating fungal development and virulence. *Microbiology and molecular biology reviews : MMBR* 64:746–85.

33. Fuller K, Rhodes J (2012) Protein Kinase A and fungal virulence: A sinister side to a conserved nutrient sensing pathway. *Virulence* 3:1–13.

34. Cullen PJ, Sprague GF (2012) The regulation of filamentous growth in yeast. *Genetics* 190:23–49.

35. Winzeler EA et al. (1999) Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. *Science (New York, N.Y.)* 285:901–6.

36. Giaever G et al. (2002) Functional profiling of the Saccharomyces cerevisiae genome. *Nature* 418:387–91.

37. Ross-Macdonald P et al. (1999) Large-scale analysis of the yeast genome by transposon tagging and gene disruption. *Nature* 402:413–8.

38. Kumar A et al. (2004) Large-scale mutagenesis of the yeast genome using a Tn7-derived multipurpose transposon. *Genome research* 14:1975–86.

39. Scherens B, Goffeau A (2004) The uses of genome-wide yeast mutant collections. *Genome biology* 5:229.

40. Huh W-K et al. (2003) Global analysis of protein localization in budding yeast. *Nature* 425:686–91.

41. Ghaemmaghami S et al. (2003) Global analysis of protein expression in yeast. *Nature* 425:737–41.

42. Gelperin DM et al. (2005) Biochemical and genetic analysis of the yeast proteome with a movable ORF collection. *Genes & development* 19:2816–26.

43. Shively CA et al. (2013) Genetic networks inducing invasive growth in Saccharomyces cerevisiae identified through systematic genome-wide overexpression. *Genetics* 193:1297–310.

44. Boundy-Mills K (2012) Yeast culture collections of the world: meeting the needs of industrial researchers. *Journal of industrial microbiology & biotechnology* 39:673–80.

45. Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Microarray. *Science* 270:467–470.

46. Gorreta F, Carbone W, Barzaghi D (2012) Genomic profiling: cDNA arrays and oligoarrays. *Methods in molecular biology (Clifton, N.J.)* 823:89–105.

47. Donatin E, Drancourt M (2012) DNA microarrays for the diagnosis of infectious diseases. *Médecine et maladies infectieuses* 42:453–9.

48. DeRisi JL, Iyer VR, Brown PO (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science (New York, N.Y.)* 278:680–6.

49. Gasch a P et al. (2000) Genomic expression programs in the response of yeast cells to environmental changes. *Molecular biology of the cell* 11:4241–57.

50. Hirasawa T, Furusawa C, Shimizu H (2010) Saccharomyces cerevisiae and DNA microarray analyses: what did we learn from it for a better understanding and exploitation of yeast biotechnology? *Applied microbiology and biotechnology* 87:391–400.

51. Menacho-Márquez M, Murguía JR (2007) Yeast on drugs: Saccharomyces cerevisiae as a tool for anticancer drug research. *Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico* 9:221–8.

52. Lorenz MC (2002) Genomic approaches to fungal pathogenicity. *Current opinion in microbiology* 5:372–8.

53. Ma J et al. (2007) An interrelationship between autophagy and filamentous growth in budding yeast. *Genetics* 177:205–14.

54. Higuchi R, Fockler C, Dollinger G, Watson R (1993) Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. *Bio/technology (Nature Publishing Company)* 11:1026–30.

55. VanGuilder HD, Vrana KE, Freeman WM (2008) Twenty-five years of quantitative PCR for gene expression analysis. *BioTechniques* 44:619–26.

56. Kubista M et al. The real-time polymerase chain reaction. *Molecular aspects of medicine* 27:95–125.

57. Marotta DH, Nantel A, Sukala L, Teubl JR, Rauceo JM (2013) Genome-Wide Transcriptional Profiling and Enrichment Mapping Reveal Divergent and Conserved Roles of Sko1 in the Candida albicans Osmotic Stress Response. *Genomics*.

58. Riera M, Mogensen E, D'Enfert C, Janbon G (2012) New regulators of biofilm development in Candida glabrata. *Research in microbiology* 163:297–307.

59. Helps C, Reeves N, Tasker S, Harbour D (2001) Use of real-time quantitative PCR to detect Chlamydophila felis infection. *Journal of clinical microbiology* 39:2675–6.

60. Ståhlberg A, Zoric N, Aman P, Kubista M (2005) Quantitative real-time PCR for cancer detection: the lymphoma case. *Expert review of molecular diagnostics* 5:221–30.

61. Sailis AD (2009) in *Real-Time PCR: Current Technology and Applications*, eds Logan J, Edwards K, Saunders N (Caister Academic Press).

62. Yeh S-H et al. (2004) Quantification and genotyping of hepatitis B virus in a single reaction by real-time PCR and melting curve analysis. *Journal of hepatology* 41:659–66.

63. Pareek CS, Smoczynski R, Tretyn A (2011) Sequencing technologies and genome sequencing. *Journal of applied genetics*:413–435.

64. Shendure J, Ji H (2008) Next-generation DNA sequencing. *Nature biotechnology* 26:1135–45.

65. Boguski MS, Tolstoshev CM, Bassett DE (1994) Gene discovery in dbEST. *Science (New York, N.Y.)* 265:1993–4.

66. Velculescu VE, Zhang L, Vogelstein B, Kinzler KW (1995) Serial analysis of gene expression. *Science (New York, N.Y.)* 270:484–7.

67. Brenner S et al. (2000) Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. *Nature biotechnology* 18:630–4.

68. Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nature reviews. Genetics* 10:57–63.

69. Mutz K-O, Heilkenbrinker A, Lönne M, Walter J-G, Stahl F (2013) Transcriptome analysis using next-generation sequencing. *Current opinion in biotechnology* 24:22–30.

70. Sittka A et al. (2008) Deep sequencing analysis of small noncoding RNA and mRNA targets of the global post-transcriptional regulator, Hfq. *PLoS genetics* 4:e1000163.

71. Kassahn KS, Waddell N, Grimmond SM (2011) Sequencing transcriptomes in toto. *Integrative biology : quantitative biosciences from nano to macro* 3:522–8.

72. Marguerat S, Bähler J (2010) RNA-seq: from technology to biology. *Cellular and molecular life sciences : CMLS* 67:569–79.

73. Nagalakshmi U et al. (2008) The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science (New York, N.Y.)* 320:1344–9.

74. Fields S (2009) Interactive learning: lessons from two hybrids over two decades. *Proteomics* 9:5209–13.

75. Toby GG, Golemis EA (2001) Using the yeast interaction trap and other two-hybrid-based approaches to study protein-protein interactions. *Methods (San Diego, Calif.)* 24:201–17.

76. Rezwan M, Auerbach D (2012) Yeast "N"-hybrid systems for protein-protein and drug-protein interaction discovery. *Methods (San Diego, Calif.)* 57:423–9.

77. Hamdi A, Colas P (2012) Yeast two-hybrid methods and their applications in drug discovery. *Trends in pharmacological sciences* 33:109–18.

78. Causier B, Davies B (2002) Analysing protein-protein interactions with the yeast two-hybrid system. *Plant molecular biology* 50:855–70.

79. Botstein D, Fink GR (2011) Yeast: an experimental organism for 21st Century biology. *Genetics* 189:695–704.

80. Yates JR (1998) Mass spectrometry and the age of the proteome. *Journal of mass spectrometry : JMS* 33:1–19.

81. Riper SK Van, Jong EP de, Carlis J V, Griffin TJ (2013) Mass spectrometry-based proteomics: basic principles and emerging technologies and directions. *Advances in experimental medicine and biology* 990:1–35.

82. Altelaar a FM, Munoz J, Heck AJR (2013) Next-generation proteomics: towards an integrative view of proteome dynamics. *Nature reviews. Genetics* 14:35–48.

83. Krogan NJ et al. (2006) Global landscape of protein complexes in the yeast Saccharomyces cerevisiae. *Nature* 440:637–43.

84. Ewing RM et al. (2007) Large-scale mapping of human protein-protein interactions by mass spectrometry. *Molecular systems biology* 3:89.

85. Alaoui-Jamali MA, Xu Y (2006) Proteomic technology for biomarker profiling in cancer: an update. *Journal of Zhejiang University SCIENCE B* 7:411–420.

86. Gygi SP et al. (1999) Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nature biotechnology* 17:994–9.

87. Bantscheff M, Schirle M, Sweetman G, Rick J, Kuster B (2007) Quantitative mass spectrometry in proteomics: a critical review. *Analytical and bioanalytical chemistry* 389:1017–31.

88. Aebersold R, Mann M (2003) Mass spectrometry-based proteomics. *Nature* 422:198–207.

89. Ong S-E (2002) Stable Isotope Labeling by Amino Acids in Cell Culture, SILAC, as a Simple and Accurate Approach to Expression Proteomics. *Molecular & Cellular Proteomics* 1:376–386.

90. Godoy LMF de et al. (2008) Comprehensive mass-spectrometry-based proteome quantification of haploid versus diploid yeast. *Nature* 455:1251–4.

91. Olsen J V et al. (2006) Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. *Cell* 127:635–48.

92. Cox J, Mann M (2011) Quantitative, high-resolution proteomics for data-driven systems biology. *Annual review of biochemistry* 80:273–99.

93. Lundby A et al. (2012) Quantitative maps of protein phosphorylation sites across 14 different rat organs and tissues. *Nature communications* 3:876.

94. Stein LD (2008) Towards a cyberinfrastructure for the biological sciences: progress, visions and challenges. *Nature reviews. Genetics* 9:678–88.

95. Casado-Vela J, Cebrián A, Gómez del Pulgar MT, Lacal JC (2011) Approaches for the study of cancer: towards the integration of genomics, proteomics and metabolomics. *Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico* 13:617–28.

96. Thorisson GA, Muilu J, Brookes AJ (2009) Genotype-phenotype databases: challenges and solutions for the post-genomic era. *Nature reviews. Genetics* 10:9–18.

97. Phan JH, Quo CF, Cheng C, Wang MD (2012) Multiscale Integration of -Omic, Imaging, and Clinical Data in Biomedical Informatics. *IEEE Reviews in Biomedical Engineering* 5:74–87.

98. Schadt EE, Linderman MD, Sorenson J, Lee L, Nolan GP (2010) Computational solutions to large-scale data management and analysis. *Nature reviews. Genetics* 11:647–57.

99. Ashburner M et al. (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nature genetics* 25:25–9.

100. Smith B et al. (2007) The OBO Foundry: coordinated evolution of ontologies to support biomedical data integration. *Nature biotechnology* 25:1251–5.

101. Berger B, Peng J, Singh M (2013) Computational solutions for omics data. *Nature reviews. Genetics* 14:333–46.

102. Khatri P, Sirota M, Butte AJ (2012) Ten years of pathway analysis: current approaches and outstanding challenges. *PLoS computational biology* 8:e1002375.

103. Lee W-P, Tzou W-S (2009) Computational methods for discovering gene networks from expression data. *Briefings in bioinformatics* 10:408–23.

104. Zhang N, Bilsland E (2011) Contributions of Saccharomyces cerevisiae to understanding mammalian gene function and therapy. *Methods in molecular biology (Clifton, N.J.)* 759:501–23.

105. Huttenhower C et al. (2009) The impact of incomplete knowledge on evaluation: an experimental benchmark for protein function prediction. *Bioinformatics (Oxford, England)* 25:2404–10.

106. Lu S, Lu X (2012) Integrating genome and functional genomics data to reveal perturbed signaling pathways in ovarian cancers. *AMIA Summits on Translational Science proceedings AMIA Summit on Translational Science* 2012:72–8.

107. Pop A, Huttenhower C, Iyer-Pascuzzi A, Benfey PN, Troyanskaya OG (2010) Integrated functional networks of process, tissue, and developmental stage specific interactions in Arabidopsis thaliana. *BMC systems biology* 4:180.

108. Judeh T, Johnson C, Kumar A, Zhu D (2013) TEAK: topology enrichment analysis framework for detecting activated biological subpathways. *Nucleic acids research* 41:1425–37.

109. Kowald A, Wierling C (2011) in *Yeast Systems Biology SE - 20*, eds Castrillo JI, Oliver SG (Humana Press), pp 345–365.

110. Menzin J et al. (2009) Mortality, length of hospitalization, and costs associated with invasive fungal infections in high-risk patients. *American journal of health-system pharmacy : AJHP : official journal of the American Society of Health-System Pharmacists* 66:1711–7.

111. Wilson LS et al. (2002) The direct cost and incidence of systemic fungal infections. *Value in health : the journal of the International Society for Pharmacoeconomics and Outcomes Research* 5:26–34.

112. Brown GD et al. (2012) Hidden killers: human fungal infections. *Science translational medicine* 4:165rv13.

113. Pfaller MA, Diekema DJ (2010) Epidemiology of invasive mycoses in North America. *Critical reviews in microbiology* 36:1–53.

114. Lo HJ et al. (1997) Nonfilamentous C. albicans mutants are avirulent. *Cell* 90:939–49.

115. Wang L, Zhai B, Lin X (2012) The Link between Morphotype Transition and Virulence in Cryptococcus neoformans. *PLoS pathogens* 8:e1002765.

116. Fortwendel JR et al. (2012) Plasma membrane localization is required for RasA-mediated polarized morphogenesis and virulence of Aspergillus fumigatus. *Eukaryotic cell* 11:966–77.

117. Gagiano M, Bauer FF, Pretorius IS (2002) The sensing of nutritional status and the relationship to filamentous growth in Saccharomyces cerevisiae. *FEMS yeast research* 2:433–70.

118. Lott TJ, Kuykendall RJ, Reiss E (1993) Nucleotide sequence analysis of the 5.8S rDNA and adjacent ITS2 region of Candida albicans and related species. *Yeast (Chichester, England)* 9:1199–206.

119. Savini V et al. (2008) Two cases of vaginitis caused by itraconazole-resistant Saccharomyces cerevisiae and a review of recently published studies. *Mycopathologia* 166:47–50.

120. Wheeler RT, Kupiec M, Magnelli P, Abeijon C, Fink GR (2003) A Saccharomyces cerevisiae mutant with increased virulence. *Proceedings of the National Academy of Sciences of the United States of America* 100:2766–70.

121. Enache-Angoulvant A, Hennequin C (2005) Invasive Saccharomyces infection: a comprehensive review. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 41:1559–68.

122. Goldstein AL, McCusker JH (2001) Development of Saccharomyces cerevisiae as a model pathogen. A system for the genetic identification of gene products required for survival in the mammalian host environment. *Genetics* 159:499–513.

123. Gimeno CJ, Ljungdahl PO, Styles C a, Fink GR (1992) Unipolar cell divisions in the yeast S. cerevisiae lead to filamentous growth: regulation by starvation and RAS. *Cell* 68:1077–90.

124. Liu H, Styles CA, Fink GR (1996) Saccharomyces cerevisiae S288C has a mutation in FLO8, a gene required for filamentous growth. *Genetics* 144:967–78.

125. Madhani HD, Fink GR (1998) The control of filamentous differentiation and virulence in fungi. *Trends in cell biology* 8:348–53.

126. Madhani HD (2000) Interplay of intrinsic and extrinsic signals in yeast differentiation. *Proceedings of the National Academy of Sciences of the United States of America* 97:13461–3.

127. Kron SJ, Gow NA (1995) Budding yeast morphogenesis: signalling, cytoskeleton and cell cycle. *Current opinion in cell biology* 7:845–55.

128. Cullen PJ, Sprague GF (2002) The roles of bud-site-selection proteins during haploid invasive growth in yeast. *Molecular biology of the cell* 13:2990–3004.

129. Pan X, Heitman J (2000) Sok2 regulates yeast pseudohyphal differentiation via a transcription factor cascade that regulates cell-cell adhesion. *Molecular and cellular biology* 20:8364–72.

130. Kuchin S, Vyas VK, Carlson M (2003) Role of the yeast Snf1 protein kinase in invasive growth. *Biochemical Society transactions* 31:175–7.

131. Cullen PJ, Sprague GF (2000) Glucose depletion causes haploid invasive growth in yeast. *Proceedings of the National Academy of Sciences of the United States of America* 97:13619–24.

132. Lorenz MC, Cutler NS, Heitman J (2000) Characterization of alcohol-induced filamentous growth in Saccharomyces cerevisiae. *Molecular biology of the cell* 11:183–99.

133. Chen H, Fink GR (2006) Feedback control of morphogenesis in fungi by aromatic alcohols. *Genes & development* 20:1150–61.

134. Gancedo JM (2001) Control of pseudohyphae formation in Saccharomyces cerevisiae. *FEMS microbiology reviews* 25:107–23.

135. Broach JR (2012) Nutritional control of growth and development in yeast. *Genetics* 192:73–105.

136. Krishna M, Narang H (2008) The complexity of mitogen-activated protein kinases (MAPKs) made simple. *Cellular and molecular life sciences : CMLS* 65:3525–44.

137. Yang S-H, Sharrocks AD, Whitmarsh AJ (2013) MAP kinase signalling cascades and transcriptional regulation. *Gene* 513:1–13.

138. Qi M, Elion EA (2005) MAP kinase pathways. *Journal of cell science* 118:3569–72.

139. Roberts CJ et al. (2000) Signaling and circuitry of multiple MAPK pathways revealed by a matrix of global gene expression profiles. *Science (New York, N.Y.)* 287:873–80.

140. Saito H (2010) Regulation of cross-talk in yeast MAPK signaling pathways. *Current opinion in microbiology* 13:677–83.

141. Cullen PJ et al. (2004) A signaling mucin at the head of the Cdc42- and MAPK-dependent filamentous growth pathway in yeast. *Genes & development* 18:1695–708.

142. Karunanithi S, Cullen PJ (2012) The Filamentous Growth MAPK Pathway Responds to Glucose Starvation Through the Mig1/2 Transcriptional Repressors in Saccharomyces cerevisiae. *Genetics*.

143. Yamamoto K, Tatebayashi K, Tanaka K, Saito H (2010) Dynamic control of yeast MAP kinase network by induced association and dissociation between the Ste50 scaffold and the Opy2 membrane anchor. *Molecular cell* 40:87–98.

144. Maeda T, Takekawa M, Saito H (1995) Activation of yeast PBS2 MAPKK by MAPKKKs or by binding of an SH3-containing osmosensor. *Science (New York, N.Y.)* 269:554–8.

145. Liu H, Styles C, Fink G (1993) Elements of the yeast pheromone response pathway required for filamentous growth of diploids. *Science* 262:1741–1744.

146. Cook JG, Bardwell L, Thorner J (1997) Inhibitory and activating functions for MAPK Kss1 in the S. cerevisiae filamentous-growth signalling pathway. *Nature* 390:85–8.

147. Madhani HD, Styles CA, Fink GR (1997) MAP kinases with distinct inhibitory functions impart signaling specificity during yeast differentiation. *Cell* 91:673–84.

148. Belotti F et al. (2012) Localization of Ras signaling complex in budding yeast. *Biochimica et biophysica acta* 1823:1208–16.

149. Zaman S, Lippman SI, Zhao X, Broach JR (2008) How Saccharomyces responds to nutrients. *Annual review of genetics* 42:27–81.

150. Stephan JS, Yeh Y-Y, Ramachandran V, Deminoff SJ, Herman PK (2010) The Tor and cAMP-dependent protein kinase signaling pathways coordinately control autophagy in Saccharomyces cerevisiae. *Autophagy* 6:294–5.

151. Pan X, Heitman J (1999) Cyclic AMP-dependent protein kinase regulates pseudohyphal differentiation in Saccharomyces cerevisiae. *Molecular and cellular biology* 19:4874–87.

152. Santangelo GM (2006) Glucose Signaling in Saccharomyces cerevisiae. *Microbiology and Molecular Biology Reviews* 70:253–282.

153. Kataoka T et al. (1984) Genetic analysis of yeast RAS1 and RAS2 genes. *Cell* 37:437–45.

154. Busti S, Coccetti P, Alberghina L, Vanoni M (2010) Glucose signaling-mediated coordination of cell growth and cell cycle in Saccharomyces cerevisiae. *Sensors (Basel, Switzerland)* 10:6195–240.

155. Thevelein JM, Voordeckers K (2009) Functioning and evolutionary significance of nutrient transceptors. *Molecular biology and evolution* 26:2407–14.

156. Lorenz MC et al. (2000) The G protein-coupled receptor gpr1 is a nutrient sensor that regulates pseudohyphal differentiation in Saccharomyces cerevisiae. *Genetics* 154:609–22.

157. Peeters T et al. (2006) Kelch-repeat proteins interacting with the Galpha protein Gpa2 bypass adenylate cyclase for direct regulation of protein kinase A in yeast. *Proceedings of the National Academy of Sciences of the United States of America* 103:13034–9.

158. Toda T, Cameron S, Sass P, Zoller M, Wigler M (1987) Three different genes in S. cerevisiae encode the catalytic subunits of the cAMP-dependent protein kinase. *Cell* 50:277–87.

159. Robertson LS, Causton HC, Young RA, Fink GR (2000) The yeast A kinases differentially regulate iron uptake and respiratory function. *Proceedings of the National Academy of Sciences of the United States of America* 97:5984–8.

160. Malcher M, Schladebeck S, Mösch H-U (2011) The Yak1 protein kinase lies at the center of a regulatory cascade affecting adhesive growth and stress resistance in Saccharomyces cerevisiae. *Genetics* 187:717–30.

161. Celenza J, Carlson M (1986) A yeast gene that is essential for release from glucose repression encodes a protein kinase. *Science* 233:1175–1180.

162. Wilson WA, Hawley SA, Hardie DG (1996) Glucose repression/derepression in budding yeast: SNF1 protein kinase is activated by phosphorylation under derepressing conditions, and this correlates with a high AMP:ATP ratio. *Current biology : CB* 6:1426–34.

163. Honigberg SM, Lee RH (1998) Snf1 kinase connects nutritional pathways controlling meiosis in Saccharomyces cerevisiae. *Molecular and cellular biology* 18:4548–55.

164. Lin SS, Manchester JK, Gordon JI (2003) Sip2, an N-myristoylated beta subunit of Snf1 kinase, regulates aging in Saccharomyces cerevisiae by affecting cellular histone kinase activity, recombination at rDNA loci, and silencing. *The Journal of biological chemistry* 278:13390–7.

165. Wang Z, Wilson WA, Fujino MA, Roach PJ (2001) Antagonistic Controls of Autophagy and Glycogen Accumulation by Snf1p, the Yeast Homolog of AMP-Activated Protein Kinase, and the Cyclin-Dependent Kinase Pho85p. *Molecular and Cellular Biology* 21:5742–5752.

166. Kuchin S, Vyas VK, Carlson M (2002) Snf1 protein kinase and the repressors Nrg1 and Nrg2 regulate FLO11, haploid invasive growth, and diploid pseudohyphal differentiation. *Molecular and cellular biology* 22:3994–4000.

167. Hong S-P, Carlson M (2007) Regulation of snf1 protein kinase in response to environmental stress. *The Journal of biological chemistry* 282:16838–45.

168. Hedbacker K, Carlson M (2008) SNF1/AMPK pathways in yeast. *Frontiers in bioscience : a journal and virtual library* 13:2408–20.

169. Vyas VK, Berkey CD, Miyao T, Carlson M (2005) Repressors Nrg1 and Nrg2 regulate a set of stress-responsive genes in Saccharomyces cerevisiae. *Eukaryotic cell* 4:1882–91.

170. Orlova M, Ozcetin H, Barrett L, Kuchin S (2010) Roles of the Snf1-activating kinases during nitrogen limitation and pseudohyphal differentiation in Saccharomyces cerevisiae. *Eukaryotic cell* 9:208–14.

171. Loewith R, Hall MN (2011) Target of rapamycin (TOR) in nutrient signaling and growth control. *Genetics* 189:1177–201.

172. Crespo JL, Hall MN (2002) Elucidating TOR signaling and rapamycin action: lessons from Saccharomyces cerevisiae. *Microbiology and molecular biology reviews : MMBR* 66:579–91, table of contents.

173. Smets B et al. (2010) Life in the midst of scarcity: adaptations to nutrient availability in Saccharomyces cerevisiae. *Current genetics* 56:1–32.

174. Thomas G, Hall MN (1997) TOR signalling and control of cell growth. *Current opinion in cell biology* 9:782–7.

175. Beck T, Hall MN (1999) The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature* 402:689–92.

176. Komeili A, Wedaman KP, O'Shea EK, Powers T (2000) Mechanism of metabolic control. Target of rapamycin signaling links nitrogen quality to the activity of the Rtg1 and Rtg3 transcription factors. *The Journal of cell biology* 151:863–78.

177. Sadeh A, Movshovich N, Volokh M, Gheber L, Aharoni A (2011) Fine-tuning of the Msn2/4-mediated yeast stress responses as revealed by systematic deletion of Msn2/4 partners. *Molecular biology of the cell* 22:3127–38.

178. Santhanam A, Hartley A, Düvel K, Broach JR, Garrett S (2004) PP2A phosphatase activity is required for stress and Tor kinase regulation of yeast stress response factor Msn2p. *Eukaryotic cell* 3:1261–71.

179. Martin DE, Hall MN (2005) The expanding TOR signaling network. *Current opinion in cell biology* 17:158–66.

180. Pascual-Ahuir A, Proft M (2007) The Sch9 kinase is a chromatin-associated transcriptional activator of osmostress-responsive genes. *The EMBO journal* 26:3098–108.

181. Cutler NS, Pan X, Heitman J, Cardenas ME (2001) The TOR signal transduction cascade controls cellular differentiation in response to nutrients. *Molecular biology of the cell* 12:4103–13.

182. Lorenz MC, Heitman J (1998) The MEP2 ammonium permease regulates pseudohyphal differentiation in Saccharomyces cerevisiae. *The EMBO journal* 17:1236–47.

183. Boeckstaens M, André B, Marini AM (2008) Distinct transport mechanisms in yeast ammonium transport/sensor proteins of the Mep/Amt/Rh family and impact on filamentation. *The Journal of biological chemistry* 283:21362–70.

184. Tate JJ, Cooper TG (2003) Tor1/2 regulation of retrograde gene expression in Saccharomyces cerevisiae derives indirectly as a consequence of alterations in ammonia metabolism. *The Journal of biological chemistry* 278:36924–33.

185. Georis I, Tate JJ, Cooper TG, Dubois E (2011) Nitrogen-responsive regulation of GATA protein family activators Gln3 and Gat1 occurs by two distinct pathways, one inhibited by rapamycin and the other by methionine sulfoximine. *The Journal of biological chemistry* 286:44897–912.

186. Neklesa TK, Davis RW (2009) A genome-wide screen for regulators of TORC1 in response to amino acid starvation reveals a conserved Npr2/3 complex. *PLoS genetics* 5:e1000515.

187. Vinod PK, Sengupta N, Bhat PJ, Venkatesh K V (2008) Integration of global signaling pathways, cAMP-PKA, MAPK and TOR in the regulation of FLO11. *PloS one* 3:e1663.

188. Pan X, Harashima T, Heitman J (2000) Signal transduction cascades regulating pseudohyphal differentiation of Saccharomyces cerevisiae. *Current opinion in microbiology* 3:567–72.

189. Gagiano M, Dyk D Van, Bauer FF, Lambrechts MG, Pretorius IS (1999) Divergent regulation of the evolutionarily closely related promoters of the Saccharomyces cerevisiae STA2 and MUC1 genes. *Journal of bacteriology* 181:6497–508.

190. Bharucha N et al. (2008) Analysis of the yeast kinome reveals a network of regulated protein localization during filamentous growth. *Mol Biol Cell* 19:2708–2717.

191. Vagnoli P, Bisson LF (1998) The SKS1 gene of Saccharomyces cerevisiae is required for long-term adaptation of snf3 null strains to low glucose. *Yeast (Chichester, England)* 14:359–69.

192. Yang Z, Bisson LF (1996) The SKS1 protein kinase is a multicopy suppressor of the snf3 mutation of Saccharomyces cerevisiae. *Yeast (Chichester, England)* 12:1407–19.

193. Gullo A (2009) Invasive fungal infections: the challenge continues. *Drugs* 69 Suppl 1:65–73.

194. Odds FC (1996) Epidemiological shifts in opportunistic and nosocomial Candida infections: mycological aspects. *International journal of antimicrobial agents* 6:141–4.

195. Bouza E, Munoz P (2008) Epidemiology of candidemia in intensive care units. *International Journal of Antimicrobial Agents* 32:S87–S91.

196. Enoch D a, Ludlam H a, Brown NM (2006) Invasive fungal infections: a review of epidemiology and management options. *Journal of medical microbiology* 55:809–18.

197. Sexton JA, Brown V, Johnston M (2007) Regulation of sugar transport and metabolism by the Candida albicans Rgt1 transcriptional repressor. *Yeast (Chichester, England)* 24:847–60.

198. Barnett JA (2008) A history of research on yeasts 12: medical yeasts part 1, Candida albicans. *Yeast (Chichester, England)* 25:385–417.

199. Hickman M a et al. (2013) The "obligate diploid" Candida albicans forms mating-competent haploids. *Nature* 494:55–9.

200. Pla J et al. (1996) Understanding Candida albicans at the molecular level. *Yeast (Chichester, England)* 12:1677–702.

201. Miranda I et al. (2013) Candida albicans CUG Mistranslation Is a Mechanism To Create Cell Surface Variation. *mBio* 4.

202. Fonzi WA, Irwin MY (1993) Isogenic strain construction and gene mapping in Candida albicans. *Genetics* 134:717–28.

203. Kim J, Sudbery P (2011) Candida albicans, a major human fungal pathogen. *Journal of microbiology (Seoul, Korea)* 49:171–7.

204. Noble SM, Johnson AD (2007) Genetics of Candida albicans, a diploid human fungal pathogen. *Annual review of genetics* 41:193–211.

205. Kumamoto CA, Vinces MD (2005) Contributions of hyphae and hypha-co-regulated genes to Candida albicans virulence. *Cellular microbiology* 7:1546–54.

206. Zheng X, Wang Y, Wang Y (2004) Hgc1, a novel hypha-specific G1 cyclin-related protein regulates Candida albicans hyphal morphogenesis. *The EMBO journal* 23:1845–56.

207. Thewes S et al. (2007) In vivo and ex vivo comparative transcriptional profiling of invasive and non-invasive Candida albicans isolates identifies genes associated with tissue invasion. *Molecular microbiology* 63:1606–28.

208. Ernst JF (2000) Regulation of dimorphism in Candida albicans. *Contributions to microbiology* 5:98–111.

209. Kinsman OS, Pitblado K, Coulson CJ (1988) Effect of mammalian steroid hormones and luteinizing hormone on the germination of Candida albicans and implications for vaginal candidosis. *Mycoses* 31:617–26.

210. Caticha O, Grover S, Winge D, Odell WD (1992) Stimulation of Candida albicans transition by human chorionic gonadotrophin and a bacterial protein. *Endocrine research* 18:133–43.

211. Brown AJ, Gow NA (1999) Regulatory networks controlling Candida albicans morphogenesis. *Trends in microbiology* 7:333–8.

212. Kadosh D, Johnson AD (2005) Induction of the Candida albicans filamentous growth program by relief of transcriptional repression: a genome-wide analysis. *Molecular biology of the cell* 16:2903–12.

213. Huang G (2012) Regulation of phenotypic transitions in the fungal pathogen Candida albicans. *Virulence* 3:251–61.

214. Verma-Gaur J, Deshpande S, Sadhale PP (2008) RAM pathway contributes to Rpb4 dependent pseudohyphal differentiation in Saccharomyces cerevisiae. *Fungal genetics and biology : FG & B* 45:1373–9.

215. King L, Butler G (1998) Ace2p, a regulator of CTS1 (chitinase) expression, affects pseudohyphal production in Saccharomyces cerevisiae. *Current genetics* 34:183–91.

216. Zhao X, Mehrabi R, Xu J-R (2007) Mitogen-activated protein kinase pathways and fungal pathogenesis. *Eukaryotic cell* 6:1701–14.

217. Saputo S, Chabrier-Rosello Y, Luca FC, Kumar A, Krysan DJ (2012) The RAM network in pathogenic fungi. *Eukaryotic cell* 11:708–17.

218. Brown PO, Botstein D (1999) Exploring the new world of the genome with DNA microarrays. *Nature genetics* 21:33–7.

219. Berman J (2006) Morphogenesis and cell cycle progression in Candida albicans. *Current opinion in microbiology* 9:595–601.

CHAPTER 2

Detecting novel pathway components of *S. cerevisiae* filamentous growth signaling

2.1. Introduction

The need to better identify pathway and subpathway topologies enriched within large-scale data sets is evident from studying the budding yeast *Saccharomyces cerevisiae*. As a prototypical eukaryote, cellular processes in yeast occur through complex sets of signaling modules. Genomic methods have been applied extensively in yeast to profile the signaling changes underlying these processes, but challenges remain toward effectively identifying pathways and subpathways within the gene lists resulting from these studies. In standard laboratory strains of *S. cerevisiae*, nutritional stress is mediated through several key signaling modules that act in concert with sets of genes responsive to a specific stimulus (1, 2). In particular, nitrogen starvation is a common form of nutrient stress for the budding yeast, and upon nitrogen stress, yeast cells initiate both general and stimulus-specific responses. Nitrogen stress initiates a general response referred to as the environmental stress response characterized by stereotypic increases and decreases in mRNA levels for ~600 genes in response to a broad set of environmental/nutritional stresses (1). Nitrogen deprivation also induces stress-specific increases in the mRNA levels of ~300 additional genes, combined with post-transcriptional regulatory mechanisms to upregulate autophagy, alter endocytosis and downregulate protein synthesis (1, 3).

Beyond the regulatory responses discussed earlier, filamentous strains of *S. cerevisiae* initiate a complex growth transition in response to nitrogen stress, forming pseudohyphal filamentous of elongated and connected cells that extend outward and downward from a yeast colony (4, 5). This filamentation is thought to be a foraging mechanism enabling non-motile yeast to scavenge for nutrients. Interestingly, related processes of hyphal development are required for virulence in the opportunistic human fungal pathogen *Candida albicans* (6, 7). In *S. cerevisiae*, the pseudohyphal growth response is regulated by at least four signaling pathways:

the target-of-rapamycin kinase network, the Ras/cAMP-dependent protein kinase A (PKA) pathway, the Snf1p kinase pathway and the Kss1p mitogen-activated protein kinase (MAPK) pathway (5, 8). The Ste12p/Tec1p transcription factor complex acts downstream of the Kss1p MAPK pathway, and the Flo8p transcription factor is phosphorylated and activated by the Tpk2p catalytic subunit of PKA (8, 9). Both factors regulate transcription of the *MUC1/FLO11* gene encoding a Glycosylphosphatidylinositol (GPI)-anchored protein important for the enhanced cell–cell adhesion observed during filamentous growth (10, 11). The filamentous growth response, however, is extensive and encompasses several other known transcriptional regulators, such as Mss11p, Phd1p and Dig1/2p, and hundreds of downstream genes and pathways (12, 13).

While these works have identified key regulatory modules that function to transduce conditions of nitrogen stress into intracellular signals that affect cell growth/shape, the full scope of the signal transductions involved in the core regulatory modules has yet to be determined. The problem is likely to be too complicated for experimental methods alone, and we believe that an integration of experimental and computational methods will be necessary to identify new subpathways within the filamentous growth network.

Thus, to detect active subpathways underlying biological processes, we utilized the innovative Topology Enrichment Analysis frameworK (TEAK), to analyze DNA microarray data profiling changes in transcript levels for the yeast genome during nitrogen stress responses. To validate that TEAK is effective in identifying biologically relevant pathways and subpathways, we analyzed transcriptional profiles of yeast responses to varying conditions of nitrogen stress (1). As a result, we identified a set of subpathways within the sphingolipid metabolic pathway that has not been phenotypically characterized previously for fitness defects during nitrogen stress, and through experimental studies, we report that the deletion of the *DPL1* and *LAG1* genes within these subpathways do confer a cell growth defect on low-nitrogen medium. Furthermore, we performed a microarray experiment and used TEAK to identify changes in mRNA levels upon deletion of two transcription factors, Flo8p and Mss11p, essential for the yeast filamentous growth response. Via TEAK we observed decreased transcript levels for a subset of genes within a subpathway involved in glycerophospholipid metabolism upon deletion of the *FLO8* and *MSS11* genes. By deletion analysis and phenotypic profiling, we report

that the *SLC1* gene within this subpathway is required for yeast filamentous growth and that its deletion results in decreased expression of a *LacZ* reporter driven by promoter elements responsive to the filamentous growth MAPK pathway. Collectively, these laboratory studies validate TEAK's utility in analyzing large-scale data sets across multiple conditions. They also indicate the broad scope of the yeast filamentous growth response for which wild-type activity of the glycerol 3-phosphate pathway for de novo biosynthesis of phosphatidic acid is required.

2.2. Materials and methods

2.2.1. Yeast strains and growth conditions

Strains used for the analysis of sphingolipid metabolism during nitrogen stress were of the non-filamentous S288c genetic background derived from BY4742 (*MATa hisΔ1 leu2Δ0 lys2Δ0 ura3Δ0*) (14). For filamentous growth studies, the strains Y825 and HLY337 were derived from the Σ1278b background (4, 15). The haploid strain Y825 has the genotype *MATa ura3-52 leu2Δ0,* and the haploid strain HLY337 has the genotype *MATa ura3-52 trp1Δ*. Standard growth media consisted of YPD prepared using 1% yeast extract, 2% bacto-peptone and 2% glucose. Synthetic media was prepared using 0.17% yeast nitrogen base (YNB) without amino acids and ammonia, 2% glucose and 5 mM ammonium sulfate (35). Hyperosmotic sensitivity was assayed using YPD supplemented with sterile 1 M Sorbitol. Nitrogen deprivation and filamentous growth phenotypes were assayed using Synthetic Low Ammonium Dextrose (SLAD) medium consisting of 0.17% YNB, 2% dextrose, 50 μM ammonium sulfate and supplemented with the necessary amino acids (16). For plates autoclaved 2% agar was added to the media.

2.2.2. Yeast gene deletions and transformations

Deletion mutants were constructed in strains Y825 and HLY337 by using a one-step polymerase chain reaction (PCR)-based gene disruption strategy (17, 18) with the G418 resistance cassette from plasmid pFA6a-KanMX6 (39). After confirming the haploid mutants via PCR, the strains were allowed to mate on YPD+G418 plates for ~20h at 30°C. Mated cells were then streaked on SC-Trp-Leu plates to select for Y825 x HLY337 diploids. Yeast

transformations were performed according to standard lithium acetate-mediated protocols (19) with modifications (20–22).

2.2.3. Microarray experiments and analysis

After culturing the yeast strains as described earlier, RNA was prepared under standard protocols using the Poly(A) Purist kit (Ambion, Austin, TX, USA). RNA concentration and purity were determined spectrophotometrically and by gel electrophoresis. Microarray hybridization was performed with the Yeast Genome S98 Array using standard protocols (Affymetrix, Inc., Santa Clara, CA, USA). All microarray experiments were performed in quadruplicate for each strain as described by (23, 24). For preprocessing the data for use by TEAK, we used GCRMA (25). To detect differentially expressed genes, we used significance analysis of microarrays (SAM) (26). For all deletion comparisons in this study, we used SAM's two-class unpaired analysis function with significance thresholds selected so that the corresponding false discovery rate was 0.

2.2.4. Growth assays

Yeast strains were inoculated in 5 ml YPD and incubated overnight at 30°C with constant shaking (250 rpm). Cultures were diluted the following morning in 5 ml YPD and allowed to grow until the culture reached an OD_{600} of ~0.6–0.8. A 1 ml aliquot of each strain was washed once with sterile water and then re-suspended in sterile water such that all cell titers were equal. Each strain was then diluted via five 10-fold serial dilutions, and 6 ml of each dilution was spotted on YPD, YPD+1 M Sorbitol, or SLAD media. The spotted plates were allowed to dry at room temperature for 15 min before being placed at 30°C for 48 h. Yeast strains were inoculated in 5 ml YPD and incubated overnight at 30°C with constant shaking (250 rpm). Cultures were back diluted the following morning in 5 ml YPD and allowed to grow until the culture reached an OD_{600} of ~0.6–0.8. A 1-ml aliquot of each strain was washed once with sterile water and re-suspended in 1 ml sterile water. Strains were triplicate loaded into either YPD or SLAD media within a 96-well plate such that the final concentration of each strain was an OD_{600} of ~0.02 (~40X dilution) in a total volume of 100 μl. Growth was measured optically every 5 min for a period of 30 h with constant shaking at 30°C in a Synergy HT Multi-Mode microplate reader

(BioTek, Winooski, VT, USA). Time point measurements represent the average of the three replicates normalized by the average of the triplicate blank controls.

2.2.5. β-Galactosidase assays for lacZ activity

Yeast strains were inoculated in 5 ml SC-URA and incubated overnight at 30°C with constant shaking (250 rpm). Cultures were diluted the following morning in 5 ml SC-URA or SLAD media and allowed to grow until the culture reached an OD_{600} of ~0.5–1. β-Galactosidase assays were performed using the Thermo Scientific Yeast β-Galactosidase Assay Kit (Thermo Fisher Scientific, Rockford, IL, USA) according to the manufacturer's protocol except that the β-galactosidase reaction was allowed to continue for a set time of 30 min for each sample before reading the absorbance. Measurements represent the average of three replicates.

2.3. Results

2.3.1. Wild-type sphingolipid metabolism is required for efficient yeast cell growth during nitrogen stress

The yeast cell response to nitrogen stress is extensive, encompassing changes at the mRNA level for hundreds of genes. Toward this point, Gasch *et al*. (1) profiled transcriptional changes in yeast upon exposure to conditions of nitrogen stress over 10 time points ranging from 30 min to 5 days. To consider specific pathways differentially regulated at the level of transcription during nitrogen stress, we analyzed these microarray data sets using TEAK for subpathways collectively showing significant changes in mRNA levels of constituent genes across the time points.

Here, we focused our studies on linear subpathways since genes within a linear subpathway are more likely to share a common phenotype and, consequently, are more amenable to genetic analysis. Via TEAK, a sphingolipid synthesis metabolic subpathway was highly ranked (within the top 25) with higher-ranking subpathways corresponding to processes that were already known to be important for cell growth during nitrogen stress (e.g. GPI-anchor biosynthesis and the yeast cell cycle). In eukaryotes, sphingolipids are an abundant membrane component, filling a structural role in membrane support and an increasingly well-studied role as

bioactive compounds involved in signal transduction; sphingolipid functions in yeast are reviewed in (27). We were particularly intrigued by the identification of the sphingolipid metabolic pathway by TEAK since previous studies have reported that the modulation of sphingolipid synthesis impacts yeast lifespan and the regulation of the yeast high osmolarity stress-responsive pathway (28, 29). Consequently, we chose to use TEAK to investigate subpathways of the sphingolipid metabolism pathway that may contribute to yeast cell survival during nitrogen stress. By sampling subpathways strictly within the sphingolipid metabolic pathway, TEAK identified a gene set encompassing *LCB3*, *LCB5*, *YDC1*, *LAG1* and *DPL1*; these genes were consistently identified in the subpathways extracted by TEAK. We subsequently analyzed these genes further for a possible role in the yeast nitrogen stress response.

The genes selected for phenotypic analysis include *LCB5*, which along with *LCB4* encode long chain base kinases that catalyze formation of the phosphorylated long chain bases (LCBPs) dihydrosphingosine 1-phosphate and phytosphingosine 1-phosphate (30). Lcb3p is a phosphatase that dephosphorylates LCBPs (31). *YDC1* encodes an alkaline ceramidase that is specific for dihydroceramide (32). Dpl1p is an LCBP lyase that cleaves LCBPs at the C_{2-3} bond to yield a long chain aldehyde and ethanolamine phosphate (33). Lag1p is a component of ceramide synthase, synthesizing ceramide from C26(acyl)-coenzyme A and dihydrosph¬ingosine or phytosphingosine (34). Interestingly, LCBs, dihydrosphingosine and phytosphingosine have been identified as important second messengers in signaling pathways that regulate cellular responses to heat stress, and LCBPs, phytoceramide and additional sphingolipid metabolic intermediates contribute to the regulation of cell growth (35, 36). It is important to note that a role in yeast cell growth under conditions of nitrogen stress had not been determined for these genes.

Consequently, we analyzed homozygous diploid yeast strains singly deleted for each of these genes in a non-filamentous genetic background to assess their ability to grow under conditions of nitrogen stress. As indicated in **Figure 2.1**, deletion of *DPL1* and *LAG1* yields a strong reduction in cell growth under conditions of nitrogen stress, both by colony assay and by spectrophotometric analysis of yeast cell growth in liquid culture. This result indicates that wild-type activity of subpathways regulating sphingolipid synthesis and catabolism is required for

efficient cell growth during conditions of nitrogen stress while validating TEAK's utility in extracting biologically relevant subpathways from large-scale data sets.

2.3.2 Transcriptional programs regulated by the filamentous growth transcription factors Flo8p and Mss11p

In filamentous strains of *S. cerevisiae*, nitrogen stress also elicits a complex morphogenetic program resulting in the transition to a filamentous form of growth. The transcription factors Flo8p and Mss11p are both required for this filamentous growth transition in that homozygous diploid *flo8Δ/Δ* and *mss11Δ/Δ* strains do not undergo filamentation under conditions of nitrogen stress (37, 38). Flo8p is phosphorylated and activated by Tpk2p, a catalytic subunit of PKA (8), and is known to directly regulate transcription from the *MUC1/FLO11* promoter (**Figure 2.2**). Flo8p-binding sites have been assessed by chromatin immunoprecipitation/microarray studies (39), indicating hundreds of target sites across the yeast genome. Collectively, the DNA recognition pattern suggests regulation of a broad set of cellular processes although the downstream effects on mRNA levels of *FLO8* deletion have not been investigated extensively. By genetic analyses, Mss11p is thought to play a central role in the yeast filamentous growth response, putatively acting in concert with both the filamentous growth MAPK and PKA pathways (37) and also acting directly on the *MUC1* promoter. Mss11p target sites have not been assessed by chromatin immunoprecipitation/microarray analysis although mRNA levels have been profiled in a haploid *mss11Δ* strain under normal growth conditions (40). In addition to their role in regulating the filamentous growth response, Flo8p and Mss11p bind cooperatively to the *STA1* gene promoter under conditions of glucose depletion, regulating the transcription of this extracellular glucoamylase important for starch degradation (41). Thus, we expect that Flo8p and Mss11p mediate extensive and diverse transcriptional programs required for wild-type filamentous growth.

Here, we used DNA microarray analysis to profile changes in mRNA levels in homozygous diploid strains of the filamentous Σ1278b genetic background deleted for *FLO8* and *MSS11*, respectively, under conditions of nitrogen stress. The results of this analysis are summarized in **Figure 2.2**. As indicated, deletion of *FLO8* resulted in decreased mRNA levels of 67 genes during nitrogen stress, and 178 genes exhibited decreased transcript abundance in the

mss11Δ/Δ strain. A smaller set of genes showing increased transcript levels were also observed in the homozygous diploid deletion strains, and these genes can be accessed from the microarray data files provided (NCBI GEO Accession Number GSE40530). By a simple over-representation approach, we analyzed the resulting gene sets for enrichment of associated gene ontology (GO) biological process terms (**Figure 2.2, Figure 2.4**). The smaller gene set exhibiting increased transcript levels showed little enrichment for informative GO terms, but the genes showing decreased mRNA levels were significantly enriched for several cellular processes involved in filamentation. From this analysis, *FLO8* deletion is likely to affect the biosynthesis/metabolism of sulfur-containing and nitrogenous compounds while also impairing wild-type transport of nitrogenous compounds. *MSS11* deletion resulted in decreased transcript levels for an overrepresented set of genes mediating cell–cell adhesion, β-glucan biosynthesis, cytoskeletal organization, and, more generally, signal transduction. These processes are known to be involved in the filamentous growth response, and the results validate the essential roles of both transcription factors during pseudohyphal development.

Interestingly, a relatively small set of genes was identified with differential mRNA levels in both the *flo8Δ/Δ* and *mss11Δ/Δ* strains (**Figure 2.4**). Within this small gene set, no GO term enrichment was observed, and the results may speak to the point that under conditions of nitrogen stress, Flo8p and Mss11p may not co-regulate an extensive set of gene targets.

2.3.3. Glycerophospholipid metabolism and the *SLC1* gene contribute to yeast pseudohyphal growth

To supplement the overrepresentation approach utilized earlier, we further analyzed the mRNA profiles of the *flo8Δ/Δ* and *mss11Δ/Δ* strains by TEAK. Here, we specifically focused on nonlinear subpathways as we expected that nonlinear relationships would be most prevalent in the highly interconnected cell processes underlying filamentous growth. The resulting TEAK analysis identified a gene set mediating glycerophospholipid metabolism as the top-ranking subpathway within the data sets (**Figure 2.3**). The corresponding GO biological process terms were not identified as being enriched in the data sets by the overrepresentation analysis. To identify a putative functional role for this subpathway within the filamentous growth response, we focused on the gene *SLC1*.

As indicated in **Figure 2.3**, *SLC1* encodes a 1-acyl-sn-glycerol-3-phosphate acetyltransferase catalyzing the acylation of *lyso*-phosphatidic acid to form the key glycerolipid biosynthesis intermediate phosphatidic acid (42). Deletion of *SLC1* does not abolish this enzymatic activity, however, as Ale1p also provides lysophospholipid acetyltransferase activity (43). Interestingly, Slc1p additionally exhibits magnesium-dependent acetyltransferase activity toward *lyso* forms of phosphatidylserine and phosphatidylinositol (42). While these functions of Slc1p are well established, its contributions toward filamentous growth had not been investigated previously, and its role, if any, in this stress response was unknown.

To determine if *SLC1* is involved in the yeast pseudohyphal response to nitrogen stress, we generated a homozygous diploid deletion of the *SLC1* gene and analyzed the resulting mutant for the ability to form surface-spread filaments under conditions of nitrogen stress. Strikingly, on low-nitrogen medium, the *slc1Δ/Δ* mutant exhibited a loss of pseudohyphal filamentation (**Figure 2.3**). To assess whether this loss of filamentous growth occurs at least in part through decreased activity of the Kss1p MAPK pathway, we introduced a plasmid-based filamentation and invasion response element (FRE)-driven *lacZ* reporter in the *slc1Δ/Δ* mutant (44). The FRE promoter sequence is recognized by the Ste12p-Tec1p transcription factor complex that acts downstream of the Kss1p MAPK pathway; thus, expression of this reporter is a good indicator of filamentous growth MAPK pathway activity. Under conditions of nitrogen stress in the *slc1Δ/Δ* mutant, we observed a significant decrease in *lacZ*-encoded β-galactosidase activity relative to wild type, indicating decreased activity of the filamentous growth MAPK pathway upon deletion of *SLC1* during nitrogen deprivation. Thus, *SLC1* and glycerophospholipid metabolism do contribute to the yeast pseudohyphal response although the mechanism of this Flo8p-and Mss11p-mediated effect needs to be elucidated further. In addition, TEAK was indeed effective in extracting biologically relevant subpathways from the transcriptional profiling data sets.

2.4. Discussion and conclusion

The identification of sphingolipid metabolic pathways as a component of the yeast nitrogen stress response is interesting given the important role of sphingolipids as second messengers. Recently, decreased sphingolipid synthesis has been reported to increase yeast cell lifespan through processes involving reduced Sch9p kinase activity and reduced chromosomal

mutations (29). More to the point, previous studies have established that inhibition of the de novo sphingolipid synthesis pathway activates the MAPK pathway mediating the yeast response to high osmolarity (the HOG pathway) (28). HOG pathway activity inhibits the yeast filamentous growth response to nitrogen stress (45, 46), which suggests that sphingolipid accumulation may be an activating signal toward pseudohyphal growth. This hypothesis can be tested in a filamentous strain of *S. cerevisiae*.

Our article focuses significantly on the pseudohyphal response to nitrogen stress in a filamentation-competent strain of *S. cerevisiae*. We specifically chose to study the filamentous growth transcription factors Flo8p and Mss11p because (i) they are required for wild-type filamentous growth; (ii) their respective transcriptional programs remain to be fully delineated; and (iii) they may function cooperatively during pseudohyphal development. By the microarray-based approach employed here, we detect changes in mRNA level that occur as both direct and indirect effects of *FLO8* and *MSS11* deletion. With respect to the transcriptional program controlled by Flo8p, the distinction between direct and indirect effects can be studied by comparing the results presented here with published chromatin immunoprecipitation/microarray analysis of Flo8p binding (39). As the latter method should be highly enriched for direct transcription factor-promoter interactions, additional transcript level changes reported here are likely to be enriched in indirect effects. Furthermore, the overlap between the data sets provides a high confidence set of Flo8p targets. In total, 15 genes were identified in common through both methods of study including the following genes required for wild-type filamentous growth: *MUC1*, *HMS1*, *NDE1*, *PDR11* and *CLN1*.

Interestingly, Flo8p and Mss11p are known to bind co-operatively to a short inverted repeat sequence in the *STA1* gene promoter under conditions of glucose limitation (41). Presumably, the transcription factors also cooperatively bind additional promoters and promoter elements. This suggests that we may observe a significant degree of overlap between the genes exhibiting differential transcript levels upon *FLO8* and *MSS11* deletion. In our analysis, however, we only observed 16 such genes in common between the two data sets. This overlapping set does include *MUC1*/*FLO11* whose promoter is known to be recognized by both factors during nitrogen stress-induced filamentous growth. In this case, though, Flo8p and Mss11p bind to Ste12p and Tec1p, which collectively recognize the FRE sequence in the *MUC1*

promoter (41). Further chromatin immunoprecipitation/sequencing studies will be helpful in determining the binding dynamics of Flo8p and Mss11p.

The transcriptional profiles of *flo8Δ* and *mss11Δ* strains indicate a broad range of cellular processes encompassed within these transcriptional programs. Although additional transcription factors contribute to the full transcriptional regulation underlying filamentous differentiation, the gene set reported here is reflective of the broad scope of this growth transition. The development of pseudohyphal filaments requires the output of complex processes regulating cell polarity, cell cycle progression, cell morphology, cell–cell adhesion, cytoskeletal organization and numerous metabolic systems. Consequently, genomics has been and continues to be very useful in identifying these higher-order processes for subsequent detailed follow-up analysis.

2.5. Acknowledgements

We thank the reviewers for their thoughtful and useful feedback.

Figure 2.1. Sphingolipid metabolism is necessary for cellular response to nitrogen stress. (**A**) A simplified representation of the sphingolipid metabolism pathway. The shaded genes were selected from TEAK for phenotypic analysis. (**B**) Colony assays of yeast cell growth in liquid culture under YPD (normal nitrogen) and SLAD (low nitrogen) across 10-fold dilutions. (**C**) Spectrophotometric analysis of yeast cell growth in liquid culture under YPD and SLAD. As seen in B and C, deletion of the genes *DPL1* and *LAG1* greatly reduces yeast cell growth under nitrogen stress.

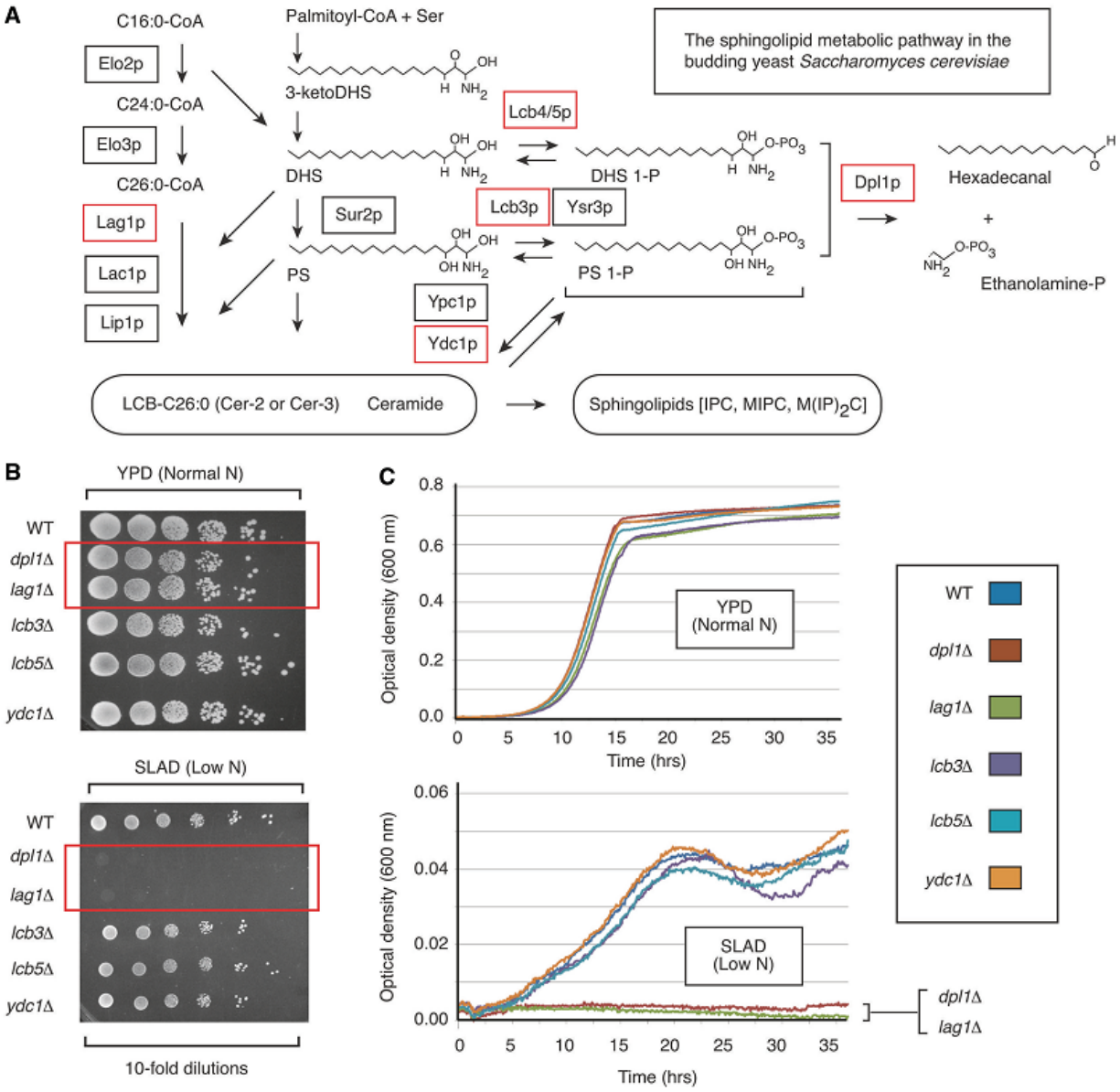


Figure 2.2. Regulation of filamentous growth by Flo8p and Mss11p. (**A**) Flo8p and Mss11p regulate the *MUC1/FLO11* promoter required for wild-type filamentous growth. (**B**) DNA microarray analysis to profile mRNA level changes in homozygous diploid strains of the filamentous Σ1278b genetic background deleted for *FLO8* and *MSS11*, respectively.

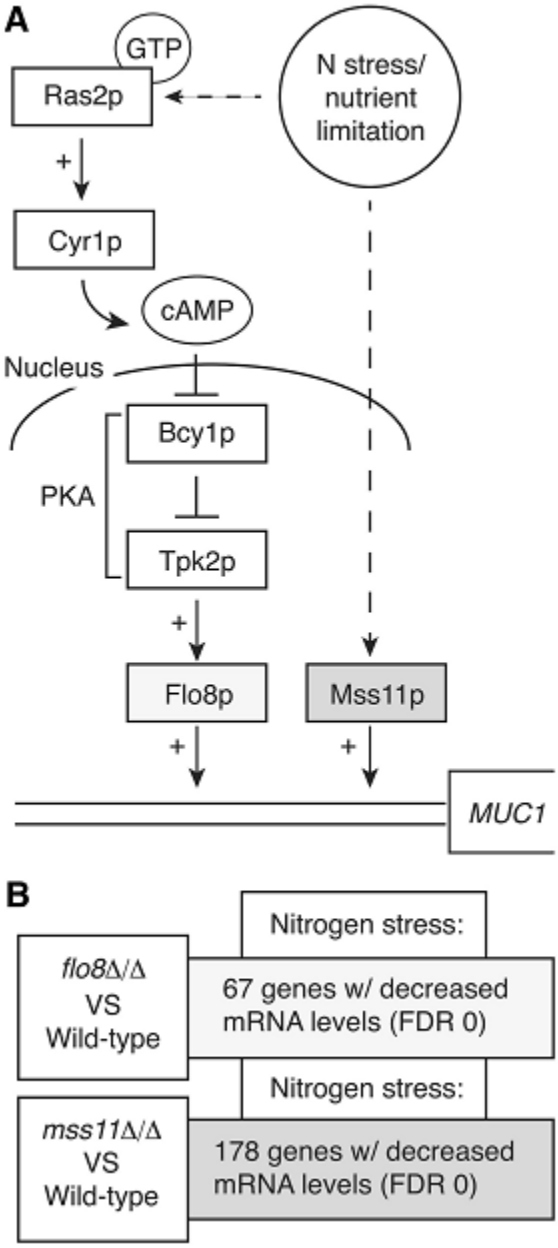


Figure 2.3. *SLC1* is necessary for cellular response to nitrogen stress and pseudohyphal growth. (**A**) A simplified representation of the glycerophospholipid metabolism pathway. (**B**) Deletion of the gene *SLC1* leads to loss of filamentous growth under SLAD growth conditions.

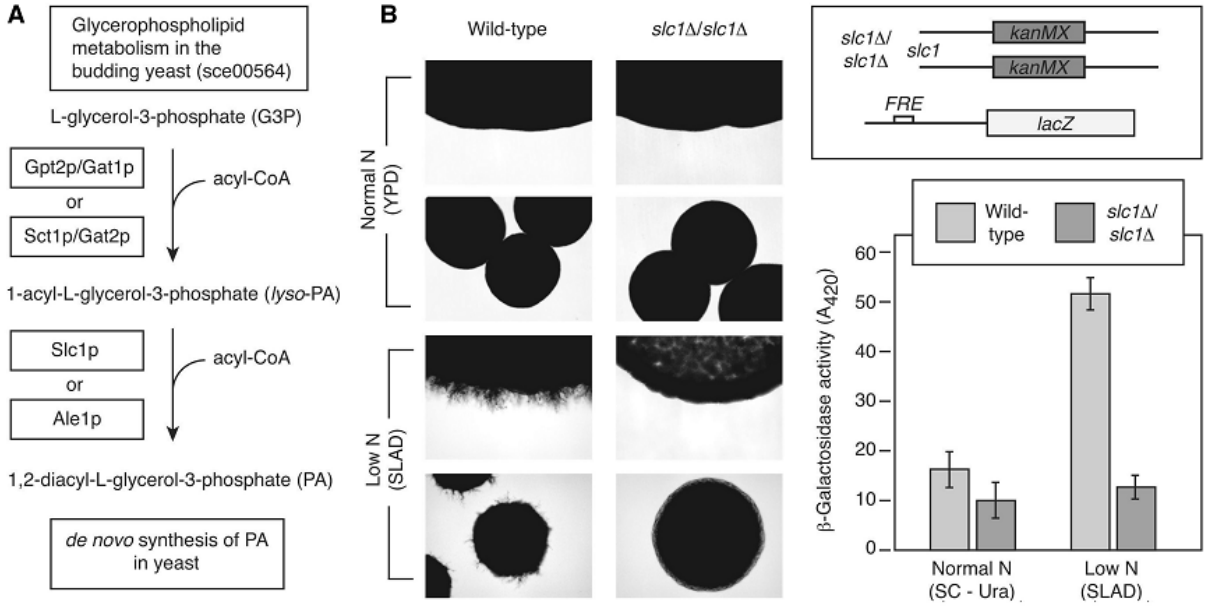
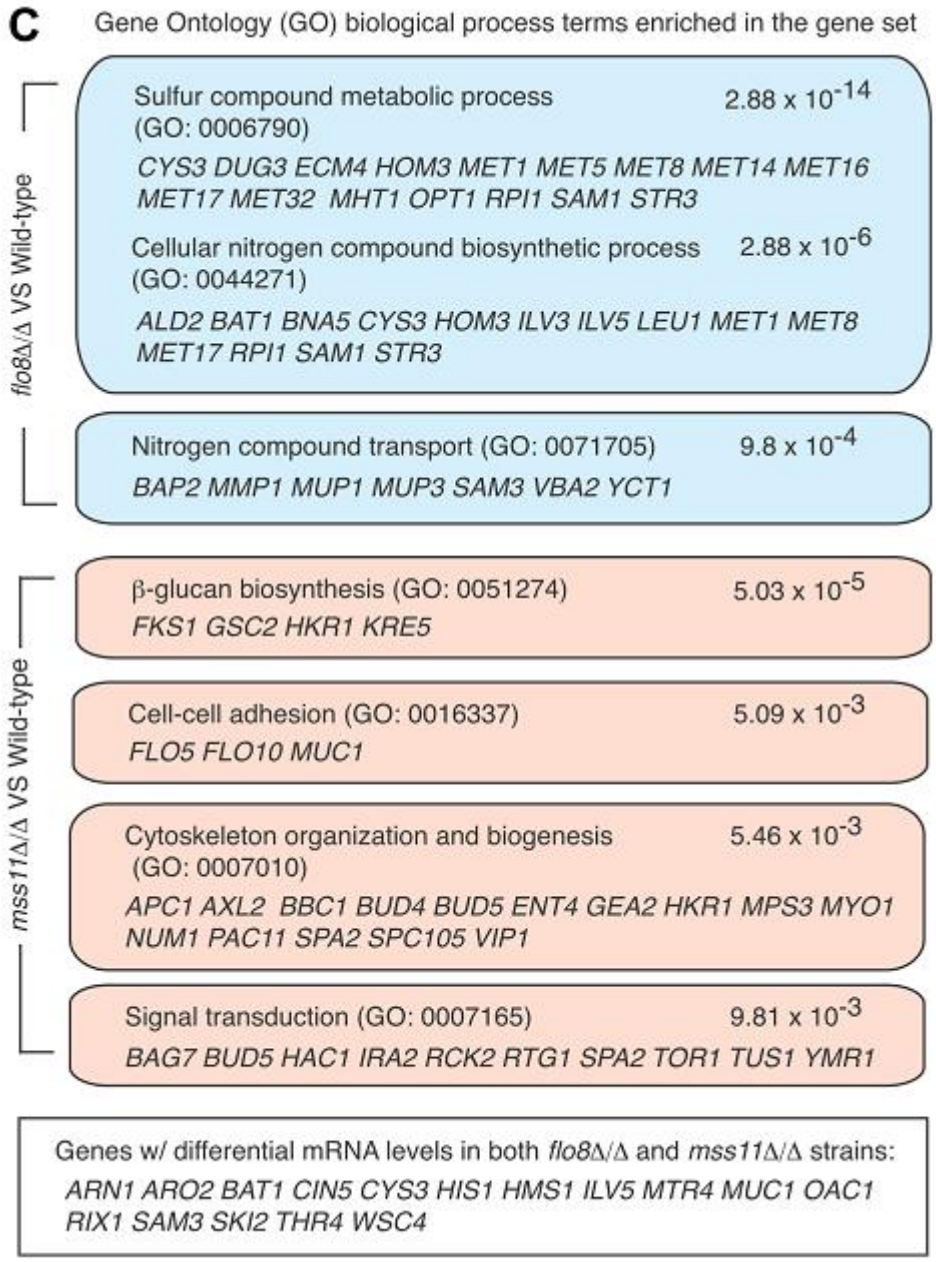


Figure 2.4. Results of a simple over-representation approach for the differentially expressed gene sets of Figure 5B in the main text. It should be noted that the *SLC1* gene discovered by TEAK is not among the results.



References

1. Gasch a P et al. (2000) Genomic expression programs in the response of yeast cells to environmental changes. *Molecular biology of the cell* 11:4241–57.

2. Causton HC et al. (2001) Remodeling of yeast genome expression in response to environmental changes. *Molecular biology of the cell* 12:323–37.

3. Mousley CJ et al. (2012) A sterol-binding protein integrates endosomal lipid metabolism with TOR signaling and nitrogen sensing. *Cell* 148:702–15.

4. Gimeno CJ, Ljungdahl PO, Styles C a, Fink GR (1992) Unipolar cell divisions in the yeast S. cerevisiae lead to filamentous growth: regulation by starvation and RAS. *Cell* 68:1077–90.

5. Roberts RL, Fink GR (1994) Elements of a single MAP kinase cascade in Saccharomyces cerevisiae mediate two developmental programs in the same cell type: mating and invasive growth. *Genes & development* 8:2974–85.

6. Braun BR, Johnson AD (1997) Control of filament formation in Candida albicans by the transcriptional repressor TUP1. *Science (New York, N.Y.)* 277:105–9.

7. Jayatilake JAMS, Samaranayake YH, Cheung LK, Samaranayake LP (2006) Quantitative evaluation of tissue invasion by wild type, hyphal and SAP mutants of Candida albicans, and non-albicans Candida species in reconstituted human oral epithelium. *Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology* 35:484–91.

8. Pan X, Heitman J (1999) Cyclic AMP-dependent protein kinase regulates pseudohyphal differentiation in Saccharomyces cerevisiae. *Molecular and cellular biology* 19:4874–87.

9. Pan X, Heitman J (2002) Protein kinase A operates a molecular switch that governs yeast pseudohyphal differentiation. *Molecular and cellular biology* 22:3981–93.

10. Guo B, Styles CA, Feng Q, Fink GR (2000) A Saccharomyces gene family involved in invasive growth, cell-cell adhesion, and mating. *Proceedings of the National Academy of Sciences of the United States of America* 97:12158–63.

11. Karunanithi S et al. (2010) Shedding of the mucin-like flocculin Flo11p reveals a new aspect of fungal adhesion regulation. *Current biology : CB* 20:1389–95.

12. Ma J et al. (2007) An interrelationship between autophagy and filamentous growth in budding yeast. *Genetics* 177:205–14.

13. Jin R, Dobry CJ, McCown PJ, Kumar A (2008) Large-scale analysis of yeast filamentous growth by systematic gene disruption and overexpression. *Mol Biol Cell* 19:284–296.

14. Giaever G et al. (2002) Functional profiling of the Saccharomyces cerevisiae genome. *Nature* 418:387–91.

15. Bharucha N et al. (2008) Analysis of the yeast kinome reveals a network of regulated protein localization during filamentous growth. *Mol Biol Cell* 19:2708–2717.

16. Lorenz MC, Heitman J (1997) Yeast pseudohyphal growth is regulated by GPA2, a G protein alpha homolog. *The EMBO journal* 16:7008–18.

17. Baudin A, Ozier-Kalogeropoulos O, Denouel A, Lacroute F, Cullin C (1993) A simple and efficient method for direct gene deletion in Saccharomyces cerevisiae. *Nucleic acids research* 21:3329–30.

18. Wach a, Brachat a, Pöhlmann R, Philippsen P (1994) New heterologous modules for classical or PCR-based gene disruptions in Saccharomyces cerevisiae. *Yeast (Chichester, England)* 10:1793–808.

19. Ito H, Fukuda Y, Murata K, Kimura A (1983) Transformation of intact yeast cells treated with alkali cations. *Journal of bacteriology* 153:163–8.

20. Coelho PS, Kumar A, Snyder M (2000) Genome-wide mutant collections: toolboxes for functional genomics. *Current opinion in microbiology* 3:309–15.

21. Kumar A, Etages SA des, Coelho PS, Roeder GS, Snyder M (2000) High-throughput methods for the large-scale analysis of gene function by transposon tagging. *Methods in enzymology* 328:550–74.

22. Kumar A, Vidan S, Snyder M (2002) Insertional mutagenesis: transposon-insertion libraries as mutagens in yeast. *Methods in enzymology* 350:219–29.

23. Ma J et al. (2007) An interrelationship between autophagy and filamentous growth in budding yeast. *Genetics* 177:205–14.

24. Ma J, Dobry CJ, Krysan DJ, Kumar A (2008) Unconventional genomic architecture in the budding yeast saccharomyces cerevisiae masks the nested antisense gene NAG1. *Eukaryotic cell* 7:1289–98.

25. Wu Z, Irizarry RA, Gentleman R, Martinez-Murillo F, Spencer F (2004) A model-based background adjustment for oligonucleotide expression arrays. *Journal of the American Statistical Association* 99:909–917.

26. Tusher VG, Tibshirani R, Chu G (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proceedings of the National Academy of Sciences of the United States of America* 98:5116–21.

27. Dickson RC (2010) Roles for sphingolipids in Saccharomyces cerevisiae. *Advances in experimental medicine and biology* 688:217–31.

28. Tanigawa M, Kihara A, Terashima M, Takahara T, Maeda T (2012) Sphingolipids regulate the yeast high-osmolarity glycerol response pathway. *Molecular and cellular biology* 32:2861–70.

29. Huang X, Liu J, Dickson RC (2012) Down-regulating sphingolipid synthesis increases yeast lifespan. *PLoS genetics* 8:e1002493.

30. Nagiec MM, Skrzypek M, Nagiec EE, Lester RL, Dickson RC (1998) The LCB4 (YOR171c) and LCB5 (YLR260w) genes of Saccharomyces encode sphingoid long chain base kinases. *The Journal of biological chemistry* 273:19437–42.

31. Qie L, Nagiec MM, Baltisberger JA, Lester RL, Dickson RC (1997) Identification of a Saccharomyces gene, LCB3, necessary for incorporation of exogenous long chain bases into sphingolipids. *The Journal of biological chemistry* 272:16110–7.

32. Mao C, Xu R, Bielawska A, Szulc ZM, Obeid LM (2000) Cloning and characterization of a Saccharomyces cerevisiae alkaline ceramidase with specificity for dihydroceramide. *The Journal of biological chemistry* 275:31369–78.

33. Saba JD, Nara F, Bielawska A, Garrett S, Hannun YA (1997) The BST1 gene of Saccharomyces cerevisiae is the sphingosine-1-phosphate lyase. *The Journal of biological chemistry* 272:26087–90.

34. D'mello NP et al. (1994) Cloning and characterization of LAG1, a longevity-assurance gene in yeast. *The Journal of biological chemistry* 269:15451–9.

35. Kim S, Fyrst H, Saba J (2000) Accumulation of phosphorylated sphingoid long chain bases results in cell growth inhibition in Saccharomyces cerevisiae. *Genetics* 156:1519–29.

36. Kolaczkowski M, Kolaczkowska A, Gaigg B, Schneiter R, Moye-Rowley WS (2004) Differential regulation of ceramide synthase components LAC1 and LAG1 in Saccharomyces cerevisiae. *Eukaryotic cell* 3:880–92.

37. Dyk D van, Pretorius IS, Bauer FF (2005) Mss11p is a central element of the regulatory network that controls FLO11 expression and invasive growth in Saccharomyces cerevisiae. *Genetics* 169:91–106.

38. Xu T et al. (2010) A profile of differentially abundant proteins at the yeast cell periphery during pseudohyphal growth. *The Journal of biological chemistry* 285:15476–88.

39. Borneman AR et al. (2006) Target hub proteins serve as master regulators of development in yeast. *Genes & development* 20:435–48.

40. Bester MC, Jacobson D, Bauer FF (2012) Many Saccharomyces cerevisiae Cell Wall Protein Encoding Genes Are Coregulated by Mss11, but Cellular Adhesion Phenotypes Appear Only Flo Protein Dependent. *G3 (Bethesda, Md.)* 2:131–41.

41. Kim TS, Kim HY, Yoon JH, Kang HS (2004) Recruitment of the Swi/Snf complex by Ste12-Tec1 promotes Flo8-Mss11-mediated activation of STA1 expression. *Molecular and cellular biology* 24:9542–56.

42. Benghezal M, Roubaty C, Veepuri V, Knudsen J, Conzelmann A (2007) SLC1 and SLC4 encode partially redundant acyl-coenzyme A 1-acylglycerol-3-phosphate O-acyltransferases of budding yeast. *The Journal of biological chemistry* 282:30845–55.

43. Jain S et al. (2007) Identification of a novel lysophospholipid acyltransferase in Saccharomyces cerevisiae. *The Journal of biological chemistry* 282:30562–9.

44. Madhani HD, Fink GR (1997) Combinatorial control required for the specificity of yeast MAPK signaling. *Science (New York, N.Y.)* 275:1314–7.

45. O'Rourke SM, Herskowitz I (1998) The Hog1 MAPK prevents cross talk between the HOG and pheromone response MAPK pathways in Saccharomyces cerevisiae. *Genes & development* 12:2874–86.

46. Pitoniak A, Birkaya B, Dionne HM, Vadaie N, Cullen PJ (2009) The signaling mucins Msb2 and Hkr1 differentially regulate the filamentation mitogen-activated protein kinase pathway and contribute to a multimodal response. *Molecular biology of the cell* 20:3101–14.

CHAPTER 3

The Yeast Sks1p Kinase Signaling Network Regulates Pseudohyphal Growth and Glucose Response

3.1. Introduction

Multiple fungal species exhibit complex morphological changes in response to environmental conditions, generating multicellular forms or structures critical to the respective life cycles of these organisms (1). These morphological transitions have been linked to virulence in several human and plant fungal pathogens, including *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigates*, and *Ustilago maydis* (2–4). In particular, several lines of study have established that the formation of hyphal filaments is required for virulence in the opportunistic human fungal pathogen *C. albicans* (5–8). The budding yeast *Saccharomyces cerevisiae* also exhibits a morphogenetic transition from its typical form to a filamentous state, and the study of this dimorphism in *S. cerevisiae* has contributed considerably to our understanding of important cell signaling mechanisms, while also providing insight into the molecular basis of fungal pathogenicity (9).

The morphological transition in *S. cerevisiae* is pronounced: yeast cells undergoing pseudohyphal growth are elongated and remain connected after cytokinesis, forming multicellular chains, or filaments (10). These filaments can spread out along the surface of a solid growth substrate as well as invade the substrate (11) and are referred to as pseudohyphae, since they resemble the hyphae of other fungal species but lack the structure of a true hyphal tube (12). Strains of *S. cerevisiae* competent to undergo pseudohyphal growth (e.g., the Σ1278b strain used here) initiate this transition in response to conditions of nitrogen stress and/or glucose deprivation (13). Consequently, pseudohyphal growth is considered to be an adaptive mechanism, enabling non-motile yeast cells to forage for nutrients when local resources become limited (14–16). The morphological changes associated with pseudohyphal growth are driven by a host of altered developmental processes, including a delay in the G2/M cell-cycle transition

that produces the elongated cell morphology (17), a switch to a unipolar budding pattern (18), and increased cell-cell adhesion (19).

At least 700 single gene deletions in the filamentous Σ1278b strain of *S.* cerevisiae result in pseudohyphal growth phenotypes (20, 21), and classical genetic studies have established three well-studied signaling pathways as regulators of pseudohyphal differentiation in the filamentous Σ1278b strain of *S. cerevisiae*: the mitogen-activated protein kinase (MAPK) pathway, the cAMP-dependent protein kinase A (PKA) pathway, and the sucrose non-fermentable (SNF) pathway (22). The yeast pseudohyphal growth MAPK pathway consists of the MAPKKK Ste11p, the MAPKK Ste7p, and the MAPK Kss1p. Ste11p is phosphorylated by the p21-activated kinase Ste20p, and Kss1p phosphorylates the key heterodimeric transcription factor Ste12p/Tec1p (23). In *S. cerevisiae*, PKA consists of the regulatory subunit Bcy1p and one of three catalytic subunits, Tpk1p, Tpk2p, and Tpk3p (24). Deletion of *TPK2* results in a loss of pseudohyphal growth, and Tpk2p has been implicated most extensively in filamentation and the response to nutrient stress (25). Tpk2p phosphorylates the transcription factor Flo8p, which is required for pseudohyphal growth (26). Snf1p is a member of the AMP-activated kinase family and regulates transcriptional changes associated with glucose derepression (27). Snf1p regulates the key pseudohyphal growth effector *FLO11* through repression of the negative regulators Nrg1p and Nrg2p (28, 29). The Kss1p MAPK pathway and PKA also activate *FLO11* transcription through Ste12p/Tec1p and Flo8p, respectively (30).

Notably, each pathway above is involved in the cellular response to nutrient availability (31–33). In particular, glucose, the preferred carbon source of budding yeast, directly activates the cAMP pathway via the G-protein coupled receptor Gpr1p (34) and the Snf1p network through the plasma membrane sensors Rgt2p and Snf3p (35). The mechanisms by which these signals are then propagated and executed to elicit pseudohyphal differentiation, however, are still under investigation. Studies from Bisson and colleagues (36, 37) identified the *SKS1* gene, encoding a Ser/Thr kinase, as a multicopy suppressor of *snf3*Δ. Yang and Bisson also demonstrated that Sks1p kinase activity was required for phenotypic suppression of *snf3*Δ. Recent work in our lab indicated that Sks1p is required for nuclear localization of the pseudohyphal growth regulators Bcy1p and Ksp1p during butanol-induced pseudohyphal growth (38). Thus, *SKS1* may regulate both glucose-responsive signaling and pseudohyphal

development, with the potential to serve as an integrator between these interrelated signaling processes.

Here, we used a combination of proteomic and genetic approaches to dissect the role of Sks1p in the relationship between glucose-responsive signaling and pseudohyphal growth. To determine the Sks1p signaling network, we implemented a mass spectrometry-based approach identifying phosphorylation events dependent upon Sks1p kinase activity. The resulting datasets identified 91 phosphophorylated residues exhibiting two-fold differential abundance after phosphopeptide enrichment in a catalytically inactive *sks1-K39R* mutant. Proteins exhibiting decreased phosphopeptide abundance upon loss of Sks1p kinase activity mapped onto interaction networks contributing to pseudohyphal growth signaling pathways and glycolysis/gluconeogenesis pathways. We identified phosphorylated residues important for cell growth under conditions of low nitrogen and low glucose and residues in the mitochondrial pyruvate dehydrogenase subunit Pda1p required for pseudohyphal growth. Epistasis studies suggest that Sks1p acts downstream of the GTP-binding protein Ras2p but upstream of PKA, and known pseudohyphal growth and glucose signaling transcription factors contribute to the establishment of wild-type *SKS1* transcript levels. *SKS1* is conserved, and by deletion analysis we find that the *C. albicans* ortholog of *SKS1*, *SHA3*, is required for wild-type colony morphology in the presence of an alternate carbon source. Collectively, the work identifies Sks1p as an important contributor to the signaling systems enabling filamentation and wild-type cellular response to glucose.

3.2. Materials and Methods

3.2.1. Yeast strains, plasmids, and growth conditions

The strains used in this study are listed in **Table 3.1** and are isogenic derivatives of the Σ1278b strain (14). All strains were generated from the MAT**a** haploid strain Y825 (*ura3-52 leu2Δ0*) and the MATα haploid strain HLY337 (*ura3-52 trp1-1*).

Standard yeast media and microbiological techniques were used (39, 40). Briefly, standard growth media consisted of YPD (1% yeast extract, 2% peptone, 2% glucose) or Synthetic Complete (SC) (0.67% yeast nitrogen base (YNB) without amino acids, 2% glucose, and 0.2% of the appropriate amino acid drop-out mix). Nitrogen deprivation and filamentous

phenotypes were assayed using Synthetic Low Ammonium Dextrose (SLAD) medium (0.17% YNB without amino acids, 2% glucose, 50μM ammonium sulfate and supplemented with appropriate amino acids) and Synthetic Low Ammonium Low Dextrose (SLALD) medium (0.17% YNB without amino acids, 0.05% glucose, 50μM ammonium sulfate and supplemented with appropriate amino acids) (41). Respiratory competency was assayed using YPG (1% yeast extract, 2% peptone, 3% glycerol). For plates, autoclaved 2% agar was added to the media.
To promote *C. albicans* vegetative growth, 80 ug/mL of uridine was added to all media unless otherwise noted. Hyphal growth was induced in *C. albicans* via growth in Spider medium and/or 10% serum-containing medium for the indicated times at 37°C (42).

Plasmids used in this study are listed in **Table 3.2**. Plasmids pFRE-lacZ and pSKS1-K39R-vYFP were constructed as described (43). To over-express *SKS1*, the *SKS1* open reading frame was amplified from genomic DNA and cloned into *XmaI-XhoI*-digested p426GPD (44). The GPD promoter was then replaced with the ADH1 promoter amplified from genomic DNA (-1464 to 0) and cloned into *SacI-XmaI*-digested p426-GPD-*SKS1* to generate plasmid pCK020.

3.2.2. Yeast gene deletions and site-directed mutagenesis

Gene deletion mutants were constructed in strains Y825 and HLY337 using a one-step PCR-based gene-disruption strategy (45, 46) with the G418 resistance cassette from plasmid pFA6a-KanMX6 (47). Integrated point mutations were generated using the one-step site-directed mutagenesis strategy described in Zheng *et al*. (48). After confirming the haploid mutants via PCR and site-directed mutants via sequencing, the strains were allowed to mate on YPD + G418 plates for approximately 20 hours at 30°C. Mated cells were then streaked on SC-Trp-Leu plates to select for Y825 x HLY337 diploids. All yeast transformations were performed according to standard lithium acetate-mediated protocols (49).

3.2.3. Surface-spread filamentation assays

Defects in surface spread filamentation were assayed as decribed in (50). In brief, yeast strains were inoculated in 5 mL SC supplemented with appropriate amino acids and incubated overnight at 30°C with constant shaking (250 rpm). Cell cultures were diluted to an OD_{600} of approximately 0.2 in fresh SC media supplemented with appropriate amino acids and grown at 30°C with shaking for at least two doublings, to an OD_{600} of approximately 0.6-1.0.

Approximately 1 mL of each cell culture was transferred into 1.7 mL microcentrifuge tubes. The cultures were washed twice with sterile H_2O before re-suspending in 1 mL sterile H_2O and serially diluting such that the density of plating was approximately10^2-10^3 cells per plate; high-density plating has been shown to decrease the rate at which cells transition to the filamentous form (51). Diluted cultures were then spread on SLAD and/or SLALD plates supplemented with appropriate amino acids and incubated at 30°C for 3 or more days. Cells were microscopically imaged using a Photometrics CoolSnapES2 digital camera mounted on a Nikon Eclipse 80i upright microscope. Colony morphology was imaged using a 4X objective while cellular morphology was imaged with a 100X oil-immersion objective.

3.2.4. Peptide sample preparation and phosphopeptide enrichment

S. cerevisiae Y825 control and sks1-K39R mutant cells were isotopically labeled with medium (Lys-4/Arg-8) amino acids during cell culture (SILAC). Cell cultures were lysed by bead beating in lysis buffer; the lysis buffer was composed of 50 mM tris buffer (pH 8.2), 8 M urea, and protease inhibitors (Roche) and phosphatase inhibitors (50 mM NaF, 50 mM beta-glycerophosphate, 1 mM sodium vanadate, 10 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride). Frozen cells were suspended in 400 µl lysis buffer and were lysed by applying three cycles of bead beating (for one minute each) with a 2-minute rest on ice between cycles. Supernatants containing protein extract were recovered by centrifugation at 14,000 g for 10 minutes, and protein concentrations were measured by Bradford assay. Equal amounts of protein from three SILAC-labeled cells were combined, treated for disulfide reduction and alkylation, and digested with TMPK-treated trypsin (Worthington Biochemical Corp., Lakewood, NJ) at a trypsin:protein ratio of 1:10 at 37 °C overnight.

Peptide mixtures were desalted with C18 (Waters) and separated into 12 strong cation exchange (SCX) fractions on a PolySulfoethyl A column (PolyLC, 150 x 4 mm) over a 48 minute salt gradient with two mobile phases: 100% solvent A (5 mM KH_2PO_4, 30% acetonitrile, pH 2.7) for 5 minutes, a linear gradient of 0-40% solvent B (250 mM KH_2PO_4, 30% acetonitrile, pH 2.7) in the following 35 min, a stiff increase of 40-100% B in 3 min, and flushing with 100% B for 5 min. Collected SCX fractions were desalted with C18 (Waters) and subjected to selective phosphopeptide enrichment using ZrO_2 (Glygen, 50 µm i.d. resin) under acidic conditions in the presence of 2,5-dihydroxy benzoic acid (52, 53). Phosphopeptides selectively

bound on ZrO_2 were eluted with 4% NH_4OH. The ZrO_2 eluate of enriched phosphopeptides and the flow-through of each SCX fraction were analyzed by nanoLC-tandem mass spectrometry (MSMS).

3.2.5. Mass spectrometric analysis and SILAC quantification

NanoLC-MSMS experiments were performed on a hybrid type mass spectrometer (Thermo, LTQ-Orbitrap XL) coupled to a nanoLC system (Eksigent, 2D nanoLC). Samples were separated on a custom capillary column (150 mm x 75 µm, 3 µm Sepax HP-C18) using a 120 min linear aqueous gradient (9-90% acetonitrile, 0.01% formic acid) delivered at 250 nL/min. The eluent was introduced on-line to the LTQ-Orbitrap via an electrospray device (Advion, TriVersa NanoMate) in positive ion mode.

The LTQ-Orbitrap was operated in a data-dependent mode alternating a full MS scan (300-1700 m/z at 60,000 resolution power at 400 m/z) in the Orbitrap analyzer and collision-induced dissociation scans (CID-MSMS) for the 7 most abundant ions with signal intensity above 500 from the previous MS scan in LTQ. Recurring precursor ions were dynamically excluded for 30 sec by applying charge-state monitoring, ions with 1 or unassigned charge states were rejected to increase the fraction of ions producing useful fragmentation. Lock mass ($[(Si(CH_3)_2O)_6]^{1+}$, m/z = 445.120029) was used for internal calibration. Each sample was analyzed by two LC-MS experiments. Raw LC-MS data file sets were processed, database searched, and quantified using MaxQuant (ver 1.0.13.8) (54) and the Mascot search engine together. Mascot database searches were performed against a composite database of forward and reverse sequences of verified yeast open reading frames from the Saccharomyces Genome Database. Variable modifications were allowed for oxidation (M) and phosphorylations (STY), as well as a fixed modification of carbamidomethylation (C). Peptide, protein, and phosphorylation site identifications were filtered at a false discover rate of 5%. The MaxQuant normalized M/L (medium/light) ratios with significance A scores less than 0.05 were considered statistically significant. 1068 peptides were identified, corresponding to 552 distinct proteins.

3.2.6. Identification of previously unreported phosphopeptides and network analysis

A network scaffold was constructed was constructed using interactions from the publicly available Kyoto Encyclopedia of Genes and Genomes (KEGG), GeneMania and BioGrid

resources (55–57). KEGG xml files for the glycolysis/gluconeogenesis (sce00010), cell cycle (sce04111), meiosis (sce04113) and MAPK signalling pathways (sce04011) were downloaded and parsed using an in-house program to create a network. The genes in the resulting network were then uploaded to GeneMania in order to retrieve additional genetic and physical interactions. Finally, the interactions for *SKS1* were downloaded from BioGrid and appended to the network. Differentially phosphorylated proteins, identified by differentially abundant phosphopeptides upon enrichment, were filtered using the significance of the medium/light isotope ratios; we implemented a significance (A) cut off at or below 0.05. The resulting protein list was mapped on to the network scaffold using Cytoscape (58). The network was clustered by node attributes to reflect the pathways from which the genes originated. As expected, the network consisted of three groups/sub-networks (glycolysis/gluconeogenesis, cell cycle/meiosis, and MAPK signaling sub-networks).

To identify novel phosphopeptides, we aggregated data from five repositories – PhosphoPep, Phosida, Phospho.ELM, PhosphoGRID, and GPMdb. Downloaded files were parsed using an in-house program, and a composite database of phosphopeptides was created. Modified peptide sequences identified in our dataset were queried against this database. To account for general ambiguity in the localization of phosphosites, we also queried the unmodified peptide sequences. These results were compiled into a list of novel phosphopeptides.

3.2.7. Assays for fitness and respiratory deficiency

Yeast strains were inoculated in 5 mL SC and incubated overnight at 30°C with constant shaking (250 rpm). Cell cultures were subsequently diluted in 6 mL of SC, SLAD, and SLALD to an OD_{600} of approximately 0.1 and incubated at 30°C with constant shaking (250 rpm) for approximately 15 hours. OD_{600} measurements were collected approximately every 3 hours from the time of dilution. Full growth curve datasets for the analysis of mutants in SC, SLAD, and SLALD media are provided in **Tables 3.5, 3.6, 3.7**, respectively. Assays for respiratory deficiency were implemented as follows. Single colonies were inoculated in 5mL YPD media and incubated with continuous agitation overnight. Cell cultures were diluted to an OD_{600} of approximately 0.3 in fresh YPD media and grown at 30°C with shaking for at least two doublings, to an OD_{600} of approximately 1.0. Each yeast cell culture was then adjusted to an identical OD_{600} and serially diluted 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}, respectively. Subsequently, 5 µL of

each diluted yeast culture was spotted onto YPD and YPG plates and incubated at 30°C for three to five days.

3.2.8. RNA preparation and qRT-PCR analysis

Yeast strains were inoculated in 5 mL SC and incubated overnight at 30°C with constant shaking (250 rpm). Cell cultures were diluted to an OD_{600} of approximately 0.3 in fresh SC, SLAD, and SLALD media and grown at 30°C with shaking for 4 hours. Afterward, cell cultures were pelleted by spinning at 3000g for 5 minutes, the supernatant was removed, and cell pellets were flash frozen in a dry ice/ethanol bath. Total RNA was extracted using the RiboPure Yeast Kit (Ambion/Life Technologies, Grand Island, NY) following the manufacturer's protocol. First-strand cDNA synthesis was performed using the Superscript II Reverse Transcriptase Kit (Invitrogen/Life Technologies, Grand Island, NY) with 2 μg of total RNA as template and Oligo $d(T)_{12-18}$ as primers according to the manufacturer's protocol. Quantitative real-time assays were performed in triplicate with a Mastercycler EP Realplex4 S (Eppendorf, Hauppauge, NY) using SYBR Green I dye-based detection (Life Technologies, Grand Island, NY). Each reaction contained 10 μL SYBR Green PCR Master Mix (Life Technologies, Grand Island, NY), 0.2 μM of the appropriate primers, and 120ng of cDNA template in a total volume of 20 μL. The real time PCR reactions were performed at 95°C for 5 minutes followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and a final step at 72°C for 30 seconds. Relative differences in RNA levels were normalized against *ACT1* levels using the delta delta C_T method (59).

3.2.9. Western analysis

Yeast strains were analyzed by Western blotting according to standard protocols (60). For Western analysis, 10 μL of protein sample were separated via SDS-PAGE and transferred to Immun-Blot PVDF (Bio-Rad, Hercules, CA) using standard methods. Protein detection was carried out using antibodies against Hemagglutinin (HA) (1:2000; Abcam, Cambridge, MA) in TBS + 0.1% Tween20 and 5% milk. After immunodetection of Hemagglutinin, the membrane was stripped using Stripping Buffer (62.5 mM Tris pH 6.8, 100mM β-mercaptoethanol, and 2% SDS) at 65°C for 30 minutes with occasional agitation. Normalization of loading was performed by probing the original membrane with antibodies against yeast 3-phosphoglycerate kinase

PGK1 (1:5000; Invitrogen/Life Technologies, Grand Island, NY) in the same buffer conditions used previously.

3.2.10. Analysis of mitochondrial morphology

Yeast strains were inoculated in 5 mL SC and incubated overnight at 30°C with constant shaking (250 rpm). Cell cultures were diluted to an OD_{600} of approximately 0.3 in fresh SC media and grown at 30°C with shaking for 4 hours. Mitochondrial morphology was scored using MitoTracker CMXR (Molecular Probes/Life Technologies, Grand Island, NY) for labeling the mitochondrial membrane and 4',6-diamidino-2-phenylindole (DAPI) for labeling mitochondrial DNA. MitoTracker was added to 1 mL aliquots of each cell culture to a final concentration of 0.5 μM, and the samples were incubated at 30°C for 30 minutes similar to Nunnari *et al.* (61). 3 μL of stained culture was then mixed with 3 μL of DAPI mounting media (9.25 mM p-Phenylenediamine (Sigma-Aldrich, St. Louis, MO), 0.18 μM DAPI (Sigma-Aldrich, St. Louis, MO), in glycerol) on a glass slide (39). The cell suspension was then covered with a glass coverslip and imaged using a Photometrics CoolSnapES2 digital camera mounted on a Nikon Eclipse 80i upright microscope. Mitochondrial morphology was imaged with a 100X oil-immersion objective using the UV filter for DAPI and TRITC filter for Mitotracker.

3.2.11. Phenotypic analysis of *C. albicans sha3Δ/SHA3*

Construction of the heterozygous *C. albicans sha3::CdHIS1/SHA3* deletion mutant was performed using the transformation methods described in Walther *et al.* (62). Wild-type and mutant colonies were grown overnight at 30°C in 3 ml YPD or SC media minus the appropriate amino acids and supplemented with uridine. To assess colony morphology, cell cultures were diluted to an OD_{600} of approximately 0.25 in fresh YPD + uri and grown at 30°C with shaking for at least two doublings, to an OD_{600} of approximately 0.6-1.0. 5 μl of each culture was spotted onto YPD + uri, YPD + uri + 10% serum, and Spider plates. After drying on the bench, YPD + uri plates were incubated at 30°C and YPD + uri + 10% serum and Spider plates were incubated at 37°C for 3-5 days.

3.3. Results

3.3.1. Sks1p kinase activity is required for pseudohyphal growth

We initially assessed the role of Sks1p in pseudohyphal growth through a series of phenotypic studies analyzing pseudohyphal filamentation in *SKS1* mutants in medium supplemented with butanol, an induction condition for pseudohyphal growth that mimics nitrogen stress (38). For this work, we constructed homozygous diploid *sks1*Δ/*sks1*Δ, and *sks1-K39R*/*sks1-K39R* mutants in the filamentous Σ1278b genetic background, with the latter strain containing a site-directed mutation yielding greatly diminished kinase activity. On low-nitrogen (SLAD) media, loss of either the gene or its kinase activity resulted in decreased surface-spread pseudohyphal filamentation relative to an isogenic wild-type strain (**Figure 3.1A**). The introduction of a centromeric plasmid bearing wildtype *SKS1* under transcriptional control of its native promoter was able to rescue the loss of pseudohyphal growth exhibited by the *sks1*Δ/Δ mutant, while introduction of a similar plasmid bearing the kinase-dead variant of *SKS1* (*sks1-K39R*) was unable to restore wild-type filamentation (**Figure 3.1B**). Over-expression of *SKS1* from a high-copy 2μ plasmid induced hyper-filamentation under conditions of nitrogen deprivation (**Figure 3.1C**). Thus, *SKS1* regulates pseudohyphal growth, as *SKS1* and Sks1p kinase activity are required for nitrogen stress-induced pseudohyphal filamentation.

3.3.2. Identification of the Sks1p signaling network

To determine the molecular basis of Sks1p kinase regulation of peudohyphal growth, we analyzed the Sks1p kinase signaling network by quantitative phosphoproteomics. Our approach was straightforward; we implemented a mass spectrometry-based method utilizing stable isotope labeling of amino acids in cell culture (SILAC) to identify proteins differentially phosphorylated upon loss of Sks1p kinase activity. As outlined in **Figure 3.2A**, a strain that was wild-type with respect to *SKS1* and an otherwise isogenic strain carrying the *sks1-K39R* allele in the filamentous Σ1278b background were made auxotrophic for arginine and lysine; the wild-type and *sks1*kinase-dead strains were subsequently grown in triplicate for five cell doublings in media containing unlabeled or $^{13}C_6$-labeled arginine and lysine, respectively, in the presence of butanol to induce pseudohyphal growth. Prepared proteins from both sets of samples were enriched for

phosphopeptides, and differences in phosphopeptide abundance between the wild-type and kinase-dead samples were determined by mass spectrometry.

By this approach, we profiled 903 phosphosites across the yeast proteome, identifying 114 phosphopeptides differentially abundant upon enrichment from the *sks1* kinase-dead strain relative to wild-type (**Figure 3.2B**). These peptides correspond to 91 proteins in total, encompassing phosphorylation events directly and indirectly dependent upon the presence of Sks1p kinase activity. Interestingly, by comparing the phosphorylation sites determined in this study against *S. cerevisiae* phosphorylation sites reported in public databases, we identified 39 new phosphorylation sites in the yeast proteome. **Table 3.3** presents novel phosphorylation sites in peptides differentially abundant upon phosphopeptide enrichment from the *sks1-K39R* mutant relative to wild type. A listing of phosphopeptides is presented in **Table 3.4**, and the full mass spectrometry dataset can be accessed at ProteomeXchange and Massive.

3.3.3. Sks1p signaling network connectivity

The set of Sks1p-dependent phosphoproteins identified in this study is statistically enriched for the cellular pathway enabling glycolysis and gluconeogenesis (**Figure 3.3.A**), as defined in the KEGG database (sce00010). To gain a better understanding of the means through which Sks1p-dependent glucose signaling impacts additional cell processes and pathways, we used this glycolysis/gluconeogenesis pathway as a starting point for the construction of an interaction network. In brief, reported genetic and physical interactions with components of the KEGG glycolysis/gluconeogenesis pathway were incorporated and expanded until Sks1p was included in the network as well as MAPK signaling and cell cycle pathways known to be required for wild-type pseudohyphal growth (**Figure 3.3**). The resulting interaction network structure indicates two points. First, the clusters of genes enriched in MAPK signaling and cell cycle control exhibit a greater number of genetic and physical interactions between each other than with the cluster of genes enriched for glycolysis/gluconeogenesis. Second, The network distance of Sks1p to proteins exhibiting Sks1p-dependent phosphorylation in the glycolysis/gluconeogenesis-enriched cluster is typically small and establishes a stronger interconnection between Sks1p and this cluster than with clusters enriched for MAPK signaling and cell cycle regulation. This result is consistent with the observed enrichment for the KEGG

glycolysis/gluconeogenesis pathway in the set of proteins exhibiting Sks1p-dependent phosphorylation.

3.3.4. Phenotypic analysis of Sks1p-dependent phosphorylation sites

As a first step towards identifying the phosphorylation events responsible for the filamentation defect observed in *sks1-K39R*, we screened a panel of yeast proteins exhibiting Sks1p-dependent phosphorylation for pseudohyphal growth gene deletion phenotypes. Genes were selected by cross-referencing the list of phosphoproteins identified by our mass spectrometry study with genes identified as having pseudohyphal growth phenotypes in Ryan *et al.* (21) and Jin *et al.* (20). For this analysis, we prioritized highly genes with a role in glucose signaling. Homozygous diploid gene deletions were generated and screened for fitness defects and surface-spread filamentation under conditions of nitrogen stress (SLAD medium) and nitrogen stress coupled with glucose stress (SLALD medium). Growth assay data can be found in **Tables 3.5-7**. Wild-type *S. cerevisiae* of the Σ1278b genetic background exhibits surface-spread filamentation on both SLAD and SLALD medium, and the homozygous *sks1*Δ/Δ mutant displays a loss of filamentation on both media. **Figure 3.4A** indicates deletion mutants exhibiting pseudohyphal growth phenotypes under conditions of nitrogen stress and nitrogen/glucose stress. Strains deleted for *BUD6*, *ITR1*, *MDS3*, *NPR3*, and *PDA1* displayed significantly decreased pseudohyphal growth on both SLAD and SLALD medium; *lrg1*Δ/Δ mutants exhibited decreased pseudohyphal growth under conditions of nitrogen stress. These genes contribute to pathways and cell processes required for pseudohyphal growth. *MDS3* and *NPR3* are TOR pathway components (63, 64), and *LRG1* encodes a putative GTPase-activating protein involved in the Pkc1p signaling pathway controlling cell wall integrity (65). Bud6p is a polarity protein required for budding (66), and Itr1p is a myo-inositol transporter (67); Pda1p is discussed further below. Also as indicated in **Figure 3.4A**, deletion of the *HXT1* gene encoding a low-affinity glucose transporter yielded hyperactive pseudohyphal growth under nitrogen and nitrogen/glucose stress. Deletion of *RCK2* encoding a kinase responsive to oxidative and osmotic stress resulted in increased pseudohyphal growth; a similar phenotype has been observed upon decreased activity of the osmo-responsive Hog1p MAPK pathway (68).

To assess the functional significance of Sks1p-dependent phosphorylation sites, we constructed homozygous diploid strains containing chromosomal point mutations in *BUD6*,

ITR1, *LRG1*, *NPR3*, and *PDA1*, substituting a non-phosphorylatable residue for the Sks1p-dependent phosphosite (**Figure 3.4.B**). Corresponding phosphopeptides for each phosphorylation site exhibited decreased abundance upon titanium dioxide enrichment in the kinase-dead *sks1-K39R* mutant. These integrated point mutants were assayed for fitness in SLAD and SLALD media, and each mutant indicated a fitness defect relative to wild-type (**Figure 3.4C and Figure 3.4D**) indicating that the mutated residues are necessary for optimal response to nitrogen and nitrogen/glucose stress.

3.3.5. Pda1p residues Y309 and S313 are necessary for pseudohyphal differentiation and respirative growth

Of the point mutants assayed above, strains with mutations in *PDA1* exhibited pseudohyphal growth defects on low-nitrogen medium. In particular, two distinct point mutations in *PDA1*, the *Y309A* and *S313A* alleles, yield a dramatic fitness defect and loss of pseudohyphal differentiation in nitrogen-limiting conditions. Pda1p is subunit of the mitochondrial pyruvate dehydrogenase complex involved in the conversion of pyruvate to acetyl-CoA during glycolysis (69). Cells lacking *PDA1* demonstrate diminished growth on glucose from a respiratory deficiency due to mitochondrial DNA loss (70). We found no noticeable difference in Pda1p protein levels for either single point-mutant (Y309A or S313A) or the double mutant (Y309A-S313A), indicating that Pda1p was actively transcribed and translated. This suggests that the mutations in Pda1p were impairing an enzymatic activity required for both optimal growth on glucose as well as pseudohyphal differentiation. Interestingly, when each mutant was screened on glycerol-containing media that forced respirative growth, the S313A mutant appeared to grow as well as wild-type, while the Y309A mutant exhibited a phenotype analogous to the *pda1Δ*/ *pda1Δ* mutant (**Figure 3.5.D**). The double Y309A-S313A mutant shared the respiration deficiency of the Y309A mutant. We also investigated whether the mutation of these residues altered mitochondrial structure or mitochondrial DNA. There appeared to be little difference in mitochondrial DNA between the wild-type strain, the *pda1*Δ/Δ mutant, or any of the site-directed mutants as measured by live cell DAPI-staining (**Figure 3.5.E, lower**). However, staining with the membrane-potential-dependent dye MitoTracker illustrates dramatic differences in mitochondrial membrane potential or structure between these mutants and wild-type (**Figure 3.5.E, middle**). The wild-type and *pda1-*

S313A strains exhibit similar mitochondrial staining, while the *pda1*Δ, *pda1-Y309A*, and *pda1-Y309A-S313A* mutants all demonstrate a mitochondrial membrane phenotype. Collectively, both the Y309A and S313A mutations result in the abolishment of pseudohyphal growth in conditions of low nitrogen, and the Y309A mutation also yields phenotypes indicative of impaired respiration.

3.3.6. Epistasis analysis of Sks1p with respect to glucose signaling and pseudohyphal growth

The studies presented here support a role for Sks1p in enabling wild-type glucose signaling and pseudohyphal growth; however, the molecular context and genetic relationships of *SKS1* with respect to both glucose/nutrient-sensing and pseudohyphal growth signaling pathways is unclear (**Figure 3.6**). Consequently, we performed epistasis experiments examining the phenotypic consequences of over-expressing *SKS1* in diploid S. cerevisiae strains deficient for components of both the nutrient-sensing and pseudohyphal signaling networks (**Figure 3.6.A** and **Figure 3.6.B**). Here, we examined whether *SKS1* could act as a high-copy suppressor of mutations in cAMP signaling (*gpr1*Δ/Δ, *ras2*Δ/Δ, and *tpk2*Δ/Δ), MAPK signaling (*ste20*Δ/Δ), or Snf1p signaling (*snf1*Δ/Δ). Each of these mutations generates a yeast strain deficient in pseudohyphal differentiation under conditions of limiting nitrogen. We found that over-expression of *SKS1* was able to suppress the *gpr1*Δ/Δ phenotype (**Figure 3.6C**). Interestingly, a *ras2*Δ/Δ mutant also demonstrated a moderate phenotypic rescue from over-expression of *SKS1*; however, *SKS1* over-expression did not restore pseudohyphal growth in a *tpk2*Δ/Δ mutant, indicating that *SKS1* acts downstream of *GPR1* and *RAS2* but upstream of *TPK2*. *SKS1* did not suppress mutations in *STE20* or *SNF1*. Consistent with this *STE20* result, the *sks1*Δ/Δ mutant exhibited no loss of pseudohyphal MAPK signaling under conditions of nitrogen limitation (data not shown), as assessed using a $P_{FRE(TEC1)}$-*lacZ* reporter system that is specifically responsive to the MAPK signaling components required for filamentous growth (43). Thus, by epsistatic analysis, Sks1p contributes to glucose-responsive cAMP signaling.

3.3.7. Mss11p and Rgt1p are involved in wild-type *SKS1* transcription

In complement to our analysis of Sks1p kinase activity, we also investigated whether known transcriptional regulators of pseudohyphal development influenced the expression of

SKS1. Analysis of *SKS1* transcription via quantitative real-time PCR identified several interesting results. First, *SKS1* mRNA levels were responsive to nitrogen and glucose deprivation in wild-type *S. cerevisiae* of the filamentous Σ1278b background. *SKS1* transcript levels increased by nearly 180% under conditions of nitrogen limitation coupled with glucose stress (SLALD medium) (**Figure 3.7A**). A comparison of *SKS1* transcript levels between mutants deleted for known transcriptional regulators of pseudohyphal differentiation (*flo8*Δ/Δ, *mfg1*Δ/Δ, *mga1*Δ/Δ, *mss11*Δ/Δ, *phd1*Δ/Δ, *phd1*Δ/Δ, and *tec1*Δ/Δ) and wild-type *S. cerevisiae* found that the transcription factor Mss11p, involved in nutrient response, invasive growth, and starch degradation, as well as Flo8p, the well-known filamentous growth transcriptional activator, exhibited minor decreases in *SKS1* mRNA levels under the indicated extracellular conditions (**Figure 3.7B**). The *flo8*Δ/Δ mutant displayed an approximate 30% reduction in *SKS1* transcript levels relative to a wild-type strain in both standard and low nitrogen/glucose SLALD media. Mss11p demonstrated a reduction in *SKS1* mRNA levels of nearly 65%, but only in standard media promoting vegetative growth. We also examined the *SKS1* transcriptional response in a diploid strain deleted for *RGT1*, encoding a glucose-responsive transcriptional regulator known to repress the expression of many *HXT* genes. Compared to the wild-type control, the *rgt1*Δ/Δ mutant demonstrated an appreciable drop in *SKS1* mRNA levels under low nitrogen conditions and a marked increase in *SKS1* transcript abundance under conditions of low nitrogen coupled with glucose limitation (**Figure 3.7B**).

3.3.8. The *SKS1* ortholog *SHA3* is required for wild-type colony morphology in *Candida albicans*

Candida albicans is both a successful commensal and pathogen of humans, sharing with *S. cerevisiae* the ability to undergo morphological transitions in response to appropriate environmental cues (71). The importance of this morphological differentiation is underscored by the fact that hyphal development is required for virulence in *C. albicans* The *S. cerevisiae SKS1* gene is conserved in *C. albicans*, and considering the strong conservation of pathway structure between these organisms, we hypothesized that the *SKS1* ortholog in *C. albicans* may serve a similar function in integrating environmental cues to regulate fungal morphology. To test this hypothesis, we generated a heterozygous deletion of the *SKS1* ortholog *SHA3* in the *C. albicans* strain BWP17. *SHA3* shares approximately 33% sequence identity with *SKS1* and also encodes a

kinase involved in glucose transport and glucose-responsive signaling (**Figure 3.8A**). On Spider growth medium in which mannitol is the carbon source, the *C. albicans SHA3* heterozygous mutant displayed an appreciable decrease in colony wrinkling relative to an isogenic wild-type strain (**Figure 3.8B**). Consistent with this result, Uhl *et al.* (72) found that a heterozygous mutant containing a transposon insertion upstream of *SHA3* in its promoter region exhibited decreased hyphal growth on Spider medium. Collectively, these data suggest a conserved role for *SKS1* and its ortholog *SHA3* in the integration of nutritional stress-responsive signaling and colony morphology.

3.4. Discussion

Cellular adaptation to nitrogen or carbon deprivation in *S. cerevisiae* requires the remodeling of cellular metabolism and the precisely coordinated restructuring of cellular morphology. Here, we identify the glucose-responsive Sks1p kinase as a signaling protein required for pseudohyphal growth induced by nitrogen stress and nitrogen stress coupled with glucose limitation. More than 92 proteins undergo Sks1p-dependent phosphorylation, and the functional scope of these phosphoproteins identifies Sks1p contribution to glucose signaling as well as additional processes and pathways required for pseudohyphal growth, including mitochondrial function. Epistasis studies indicate that *SKS1* acts downstream of *GPR1* and *RAS2*, consistent with Sks1p regulation of or by glucose-responsive cAMP signaling. *SKS1* transcript levels are dependent upon Mss11p and Rgt1p. *SKS1* is conserved, and the *SKS1* ortholog *SHA3* in *C. albicans* is required for wild-type colony morphology under conditions of glucose limitation with mannitol as a carbon source. Collectively, these results are consistent with a function for Sks1p kinase activity in the integration of glucose-responsive signaling and filamentous development – an example of signaling crosstalk that has not been extensively studied or well understood.

In this study, we utilized a SILAC-based mass spectrometry approach to identify phosphorylation events dependent upon Sks1p kinase activity. In *S. cerevisiae*, several phosphoproteomic strategies have been utilized to profile differential phosphorylation (73–75). In particular, Bodenmiller *et al.* (76) recently implemented a label-free mass-spectrometry approach to investigate the global phosphoproteomic response of *S. cerevisiae* to the systematic deletion of protein kinases and phosphatases. Trade-offs exist in considering the relative

advantages of both label-free and labeling strategies. Label-free methods have been shown to identify a larger number of proteins than label-based methods; however, SILAC-based strategies typically enable better quantification and identification of differentially abundant proteins, while also providing greater reproducibility across samples (76). It is important to bear in mind that both label-free and SILAC-based interventional phosphoproteomic methods identify direct and indirect phosphorylation events; consequently, the studies here are intended to identify the broad scope of cell processes and pathways encompassed within the Sks1p signaling network.

Notably, the study by Bodenmiller and colleagues did address the Sks1p signaling network in a non-filamentous strain under vegetative growth conditions, and approximately 30% of the proteins detected in this analysis were also identified by label-free methods in that work. Further, the overlap between the datasets is striking, in that nine proteins exhibiting Sks1p-dependent phosphorylation in a non-filamentous strain under vegetative conditions were also identified as being differentially phosphorylated in our analysis of *sks1-K39R* in a filamentous strain under conditions inducing pseudohyphal growth; these phosphoproteins include Cdc37p, Crp1p, Fyv8p, Hxt1p, Mrh1p, Mtc1p, Pda1p, Pil1p, Ptr2p, Rck2p, Zuo1p, and Ymr196w. The proteins Hxt1p, Rck2p, and Pil1p are stress-responsive, and Mrh1p, Pil1p, and Pda1p have been reported to localize to mitochondria, highlighting important processes and functions required for wild-type glucose signaling and pseudohyphal growth.

The dependence of Pda1p phosphorylation upon Sks1p and phenotypic analysis of Pda1 Y309A and S313A mutants underscores that wild-type mitochondrial membrane structure and function is interconnected with Sks1p kinase signaling. Interestingly, signaling pathways that regulate filamentation, cAMP-PKA and Snf1p, have also been shown to target mitochondria (77–79), and genetic screens of pseudohyphal deficient mutants have identified genes required for mitochondrial function (80). The S313 residue of Pda1p is a known phosphorylation site, and in a non-filamentous strain of *S. cerevisiae*, Pda1p was inactivated by Ser313 phosphorylation (81). Consistent with that result, the *S313A* mutant we constructed in the filamentous Σ1278b background did not exhibit a growth defect on medium with glycerol as the sole carbon source. Interestingly, however, both the Pda1p S313 and Y309 residues are required for pseudohyphal growth. Sks1p is a Ser/Thr kinase; consequently the Y309 residue in Pda1p is not expected to be a direct phosphorylation target of Sks1p. Ongoing investigations are directed towards identifying

the kinase that phosphorylates Pda1p Y309 and the mechanism by which the Y309 and S313 residues contribute to pseudohyphal growth.

Our results indicate that *SKS1* mRNA levels are glucose-regulated in the filamentous Σ1278b strain and that this regulation in SLALD medium is carried out in part by Rgt1p. Two lines of evidence support this result. First, analysis of the yeast transcriptional response to glucose by Wang *et al.* (82) indicated that *SKS1* mRNA levels increase more than two-fold when cells are switched from galactose to glucose-containing media. Second, over-expression of the *SKS1* promoter sequence can partially rescue the *snf3*Δ phenotype presumably by titration of Rgt1p (83). In addition to possessing binding sites for Rgt1p, the *SKS1* promoter is reportedly bound by Mss11p and Flo8p (21), although we observe that the relative individual contributions of these transcription factors to the establishment of *SKS1* mRNA levels is modest under conditions of nitrogen limitation and nitrogen/glucose-stress. Considered collectively, transcriptional regulation of *SKS1* likely results from the combinatorial contributions of numerous transcription factors. Under conditions of glucose limitation, Rgt1p actively binds target promoters to repress transcription of glucose-induced genes, and the observed increase in *SKS1* transcript levels upon *RGT1* deletion under low-glucose conditions is consistent with this observation. However, *SKS1* mRNA levels increase upon growth in SLALD media, indicating that Rgt1p cannot be predominantly responsible for the establishment of overall *SKS1* transcript levels. Additional factors, including Mss11p and Flo8p, must contribute to this transcriptional control as well, presenting a more complex picture of *SKS1* transcriptional control.

Coupling findings from this study with previous work, we suggest that Sks1p mediates cellular response to glucose limitation and nitrogen stress by signaling through Gpr1p and the cAMP-PKA pathway. Early studies from Bisson and colleagues (84) indicated that *SKS1* suppression of *snf3*Δ acted independently of previously identified *snf3*Δ genomic suppressors, *ssn6*Δ, *rgt1*Δ, and *RGT2*, consistent with involvement in a pathway independent of the Snf3p/Rgt2p glucose-sensing system. In this study, we demonstrate that *SKS1* is a high-copy suppressor of pseudohyphal-deficient *gpr1*Δ/Δ and *ras2*Δ/Δ mutants. Both Gpr1p and Ras2 are components of the cAMP-dependent PKA pathway. Gpr1p is a nutrient sensor that activates cAMP in response to low-levels of extracellular glucose (34) and regulates pseudohyphal differentiation in *S. cerevisiae* (85). Over-expression of *SKS1* failed to restore pseudohyphal growth to a strain deleted for *TPK2*. Tk2p is one of three catalytic subunits of PKA; *TPK2* is

required for pseudohyphal growth, and its function is required for the phosphorylation of Flo8p and additional key signaling events necessary for pseudohyphal differentiation (86). Thus, Sks1p may contribute to the regulation of Tpk2p or may be regulated indirectly by Tpk1p or Tpk3p. Sks1p has not been identified as a phosphoprotein to date, and no such mechanism of Sks1p regulation have been reported.

Pseudohyphal growth in *S. cerevisiae* is an excellent model of related processes of filamentous development in the principal opportunistic human fungal pathogen *Candida albicans*. In *C. albicans*, a variety of culture conditions, including growth on Spider medium with mannitol as a carbon source, results in the development of pseudohyphae and true hyphal tubes (7). Orthologs of many *S. cerevisiae* pseudohyphal growth genes play similarly important roles in *C. albicans* hyphal development, and we find that the *SKS1* ortholog *SHA3* is required for wild-type colony morphology in *C. albicans* on Spider medium. The cAMP-PKA pathway is required for hyphal development and virulence in *C. albicans*, exhibiting structural similarity to the orthologous pathway in *S. cerevisiae*. Notably, *GPR1* is conserved, and Ras1p in *C. albicans* contributes to the production of cAMP through adenylate cyclase in response to various stimuli (87). The PKA catalytic subunits Tpk1p and Tpk2p have been identified in *C. albicans*, and it will be interesting to determine if the functional relationship between *SHA3* and this cAMP-PKA pathway is similar to that which we observe in *S. cerevisiae*. With the interrelationship between *C. albicans* morphogenesis/hyphal development and virulence, Sha3p holds relevance in understanding fungal pathogenicity.

3.5. Acknowledgements

We thank Mark Johnston, Kobi Simpson, Jason E. Gestwicki, and Daniel J. Klionsky for reagents and/or helpful discussions regarding this manuscript.

Figure 3.1. Phenotypic analysis of Sks1p mutants in the filamentous Σ1278b genetic background under conditions of nitrogen deprivation. (A) Diploid *sks1Δ/sks1Δ* and diploid *sks1-K39R/sks1-K39R* kinase dead mutants demonstrate a loss of pseudohyphal development as compared to the wildtype. (B) Addition of a centromeric plasmid bearing wildtype *SKS1* to the *sks1Δ/sks1Δ* deletion mutant restores pseudohyphal growth, while addition of the same plasmid bearing the kinase dead *sks1-K39R* allele fails to recover. (C) Over-expression of *SKS1* from a 2μ plasmid induces hyper-filamentation in wildtype yeast.

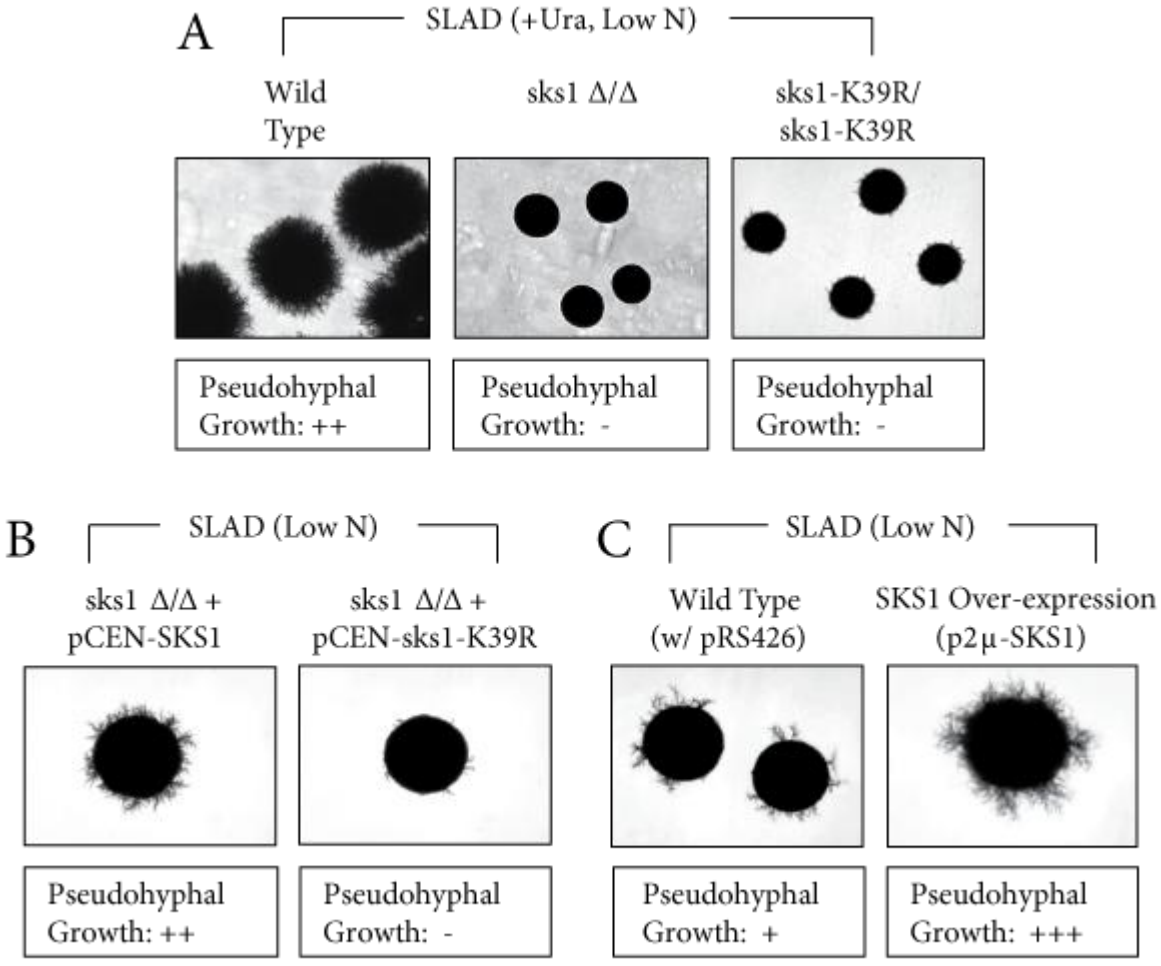
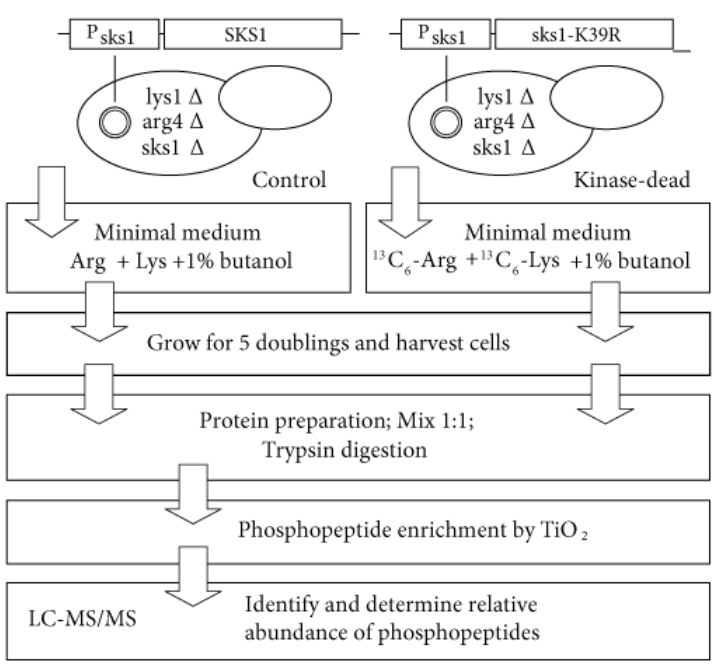


Figure 3.2. Phosphoproteomic analysis of Sks1p kinase in the filamentous Σ1278b genetic background. (A) Schematic overview of the SILAC analysis of Sks1p kinase activity. (B) Subset of proteins demonstrating a decreased or increased abundance of phosphorylation in the *sks1-K39R* kinase dead strain relative to wildtype.

A



B Phosphosites identified (localized and quantified): 903

Phosphosites with decreased abundance in sks1-K39R: 62
Corresponding proteins: 50

Ade4	Dcp2					
Ato2	Dna2	Gly1	Itr1	Pno1	Sfl1	Vba4
Bap2	Dre2	Hal5	Leu1	Prb1	Shs1	Ycr061w
Bfr2	Dus4	Hnm1	Lrg1	Prp43	Sla2	Yjl213w
Bna5	Fol2	Hsp60	Mds3	Ptr2	Tom20	Ymr196w
Bud6	Fps1	Hxt1	Npr3	Rbs1	Tpo4	Yor1
Cdc16	Fyv8	Ina1	Pda1	Rck2	Trs120	Zuo1
Crp1	Glc8	Irc5	Pdr5	Rrp36	Ubc6	

Phosphopeptides with increased abundance in sks1-K39R: 52
Corresponding proteins: 41

Abp1	Cdc37	Hsp26	Pat1	Ras2	Stm1	Ykr018c
Adh1	Cue4	Hsp42	Pbi2	Rfc5	Tom70	Ylr257w
Amd1	Cue5	Mrh1	Pfk1	Sbp1	Tsl1	Ynl035c
Are2	Dps1	Mtc1	Pil1	Sec21	Yct1	Ypk2
Atg33	Gcs1	Om45	Pma1	Sec31	Yel043w	Yro2
Cdc33	Gsy2	Osh2	Ptk2	Ssd1	Ygr250c	

Figure 3.3. Regulatory network connectivity of Sks1p signaling identified via phosphoproteomic analysis. (A) Macroscopic view of select Sks1p signaling proteins involved in glycolysis, cell cycle, and MAPK signaling. Numbers indicated specific proteins demonstrating changes in peptide phosphorylation in the *sks1-K39R* kinase dead strain. (B) Sks1p signaling proteins identified within glycolysis/gluconeogenesis (red). (C) Sks1p signaling proteins identified within cell cycle/cell division cluster (red).

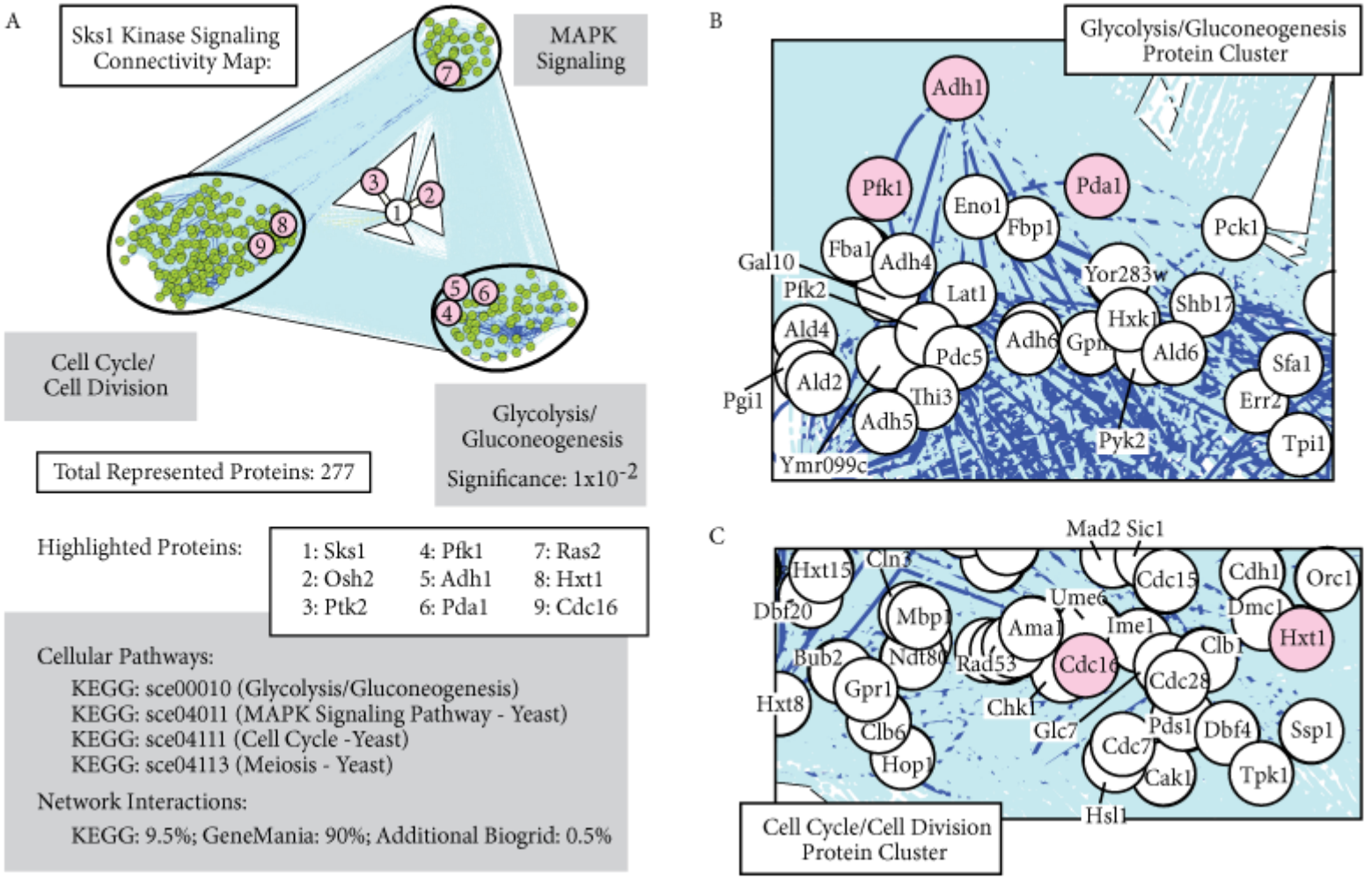
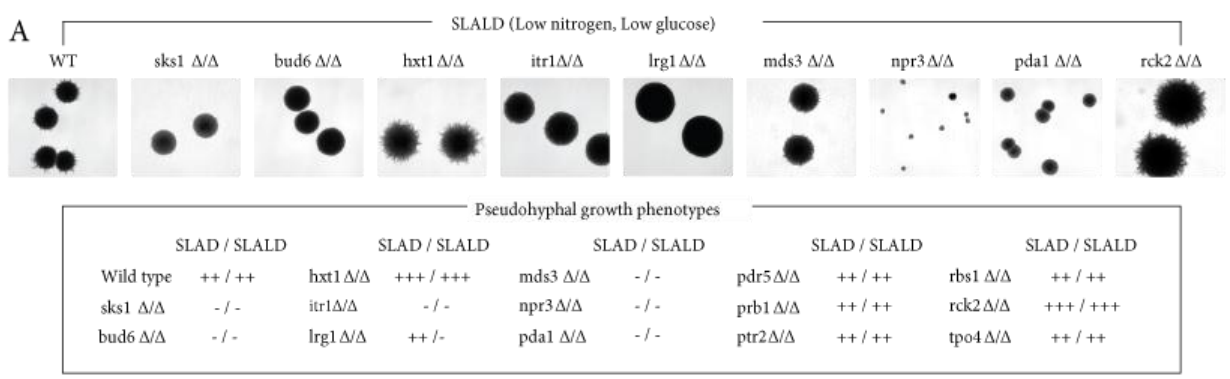


Figure 3.4. Phenotypic analysis of diploid mutants for proteins exhibiting 2-fold or greater loss of phosphorylation in *sks1-K39R* mutant background. (A) Several diploid deletion mutants exhibited changes in pseudohyphal development compared to wild type under low nitrogen and low nitrogen, low glucose conditions. (B) A subset of the proteins that demonstrated a change in pseudohyphal development where chosen for functional analysis of their identified phosphosite(s). All diploid, integrated point mutants demonstrated a significant fitness defect compared to wildtype when grown in liquid media under nitrogen limiting or nitrogen and glucose limiting conditions as indicated by the long-term cell titer (C) and growth curves (D) for each condition.



B

Protein	Phosphopeptide	sks1-K39R/ WT
Bud6	_RLSSVVTTSPDK_ (347)	0.44
Itr1	_TSQSNVGDAVGNAD SVEFNSEHDSPSK_ (26, 605)	0.37
Lrg1	_ENVESNTK_ (605)	0.046
Npr3	_VNSGEADTVYDSN IGHEASTDTPNLR_ (486)	0.43
Pda1	_YGGHSMSDPGTTYR_ (309, 313)	0.50

C

Strain	SLAD (14.5 hrs) OD_{660}	SLALD (14.5 hrs) OD_{660}
Wild Type	7.41 ± 0.21	3.34 ± 0.06
bud6-S347A/ bud6-S347A	1.18 ± 0.03	0.84 ± 0.05
itr1-S26A/ itr1-S26A	1.75 ± 0.07	1.01 ± 0.04
lrg1-S605A/ lrg1-S605A	1.30 ± 0.01	0.85 ± 0.04
npr3-S486A/ npr3-S486A	1.04 ± 0.02	0.72 ± 0.09
pda1-Y309A/ pda1-Y309A	1.12 ± 0.01	0.35 ± 0.04
pda1-S313A/ pda1-S313A	1.04 ± 0.01	0.74 ± 0.09
pda1-Y309A S313A/ pda1-Y309A S313A	1.39 ± 0.16	0.73 ± 0.02

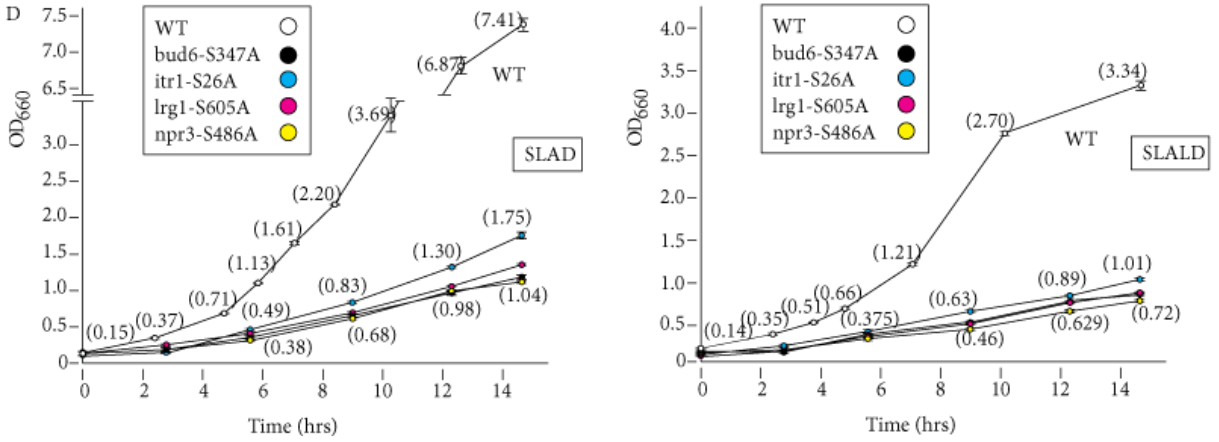


Figure 3.5. Phenotypic analysis of PDA1 point mutants. (A) Both diploid mutant strains, *pda1-Y309A* and *pda1-S313A*, demonstrated a marked decrease in pseudohyphal differentiation on nitrogen limited media. (B) The *pda1-Y309A* and *pda1-S313A* mutant strains exhibited a significant fitness defect when grown in low-nitrogen liquid media. (C) Western analysis of PDA1 mutants showed formation of PDA1 at wildtype levels. (D) Growth of all *PDA1* mutants on both fermentable (YPD) and non-fermentable media (YPGlycerol) indicate a respirative defect in the *pda1Δ/pda1Δ*, the *pda1-Y309A*, and the *pda1-Y309A-S313A* mutants. (E) The mitochondrial content and membrane-potential-based structure was imaged using DAPI and MitoTracker, respectively. Minimal changes were observed in mitochondrial content (DAPI stain, bottom row), while specific morphological/membrane-potential defects were observed in the *pda1Δ/pda1Δ*, the *pda1-Y309A*, and the *pda1-Y309A-S313A* mutants (MitoTracker, middle row).

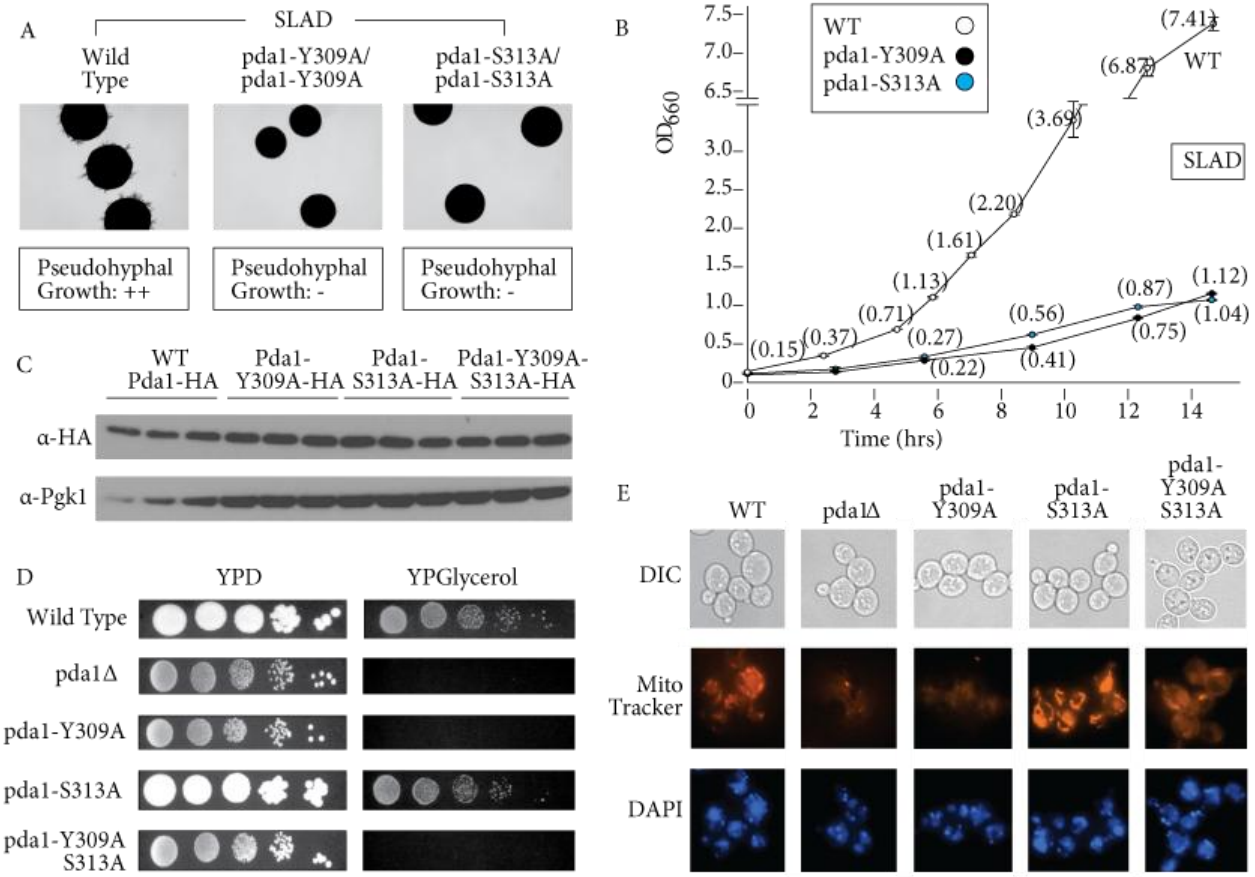
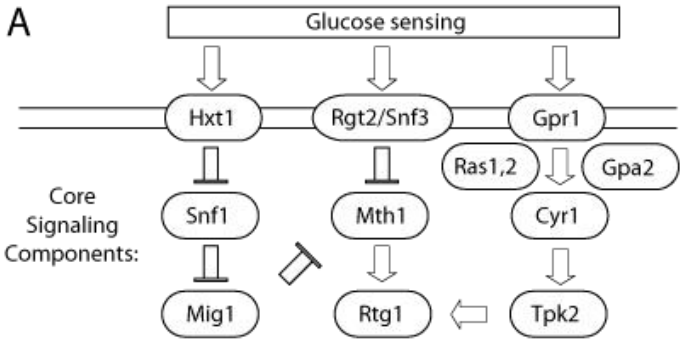
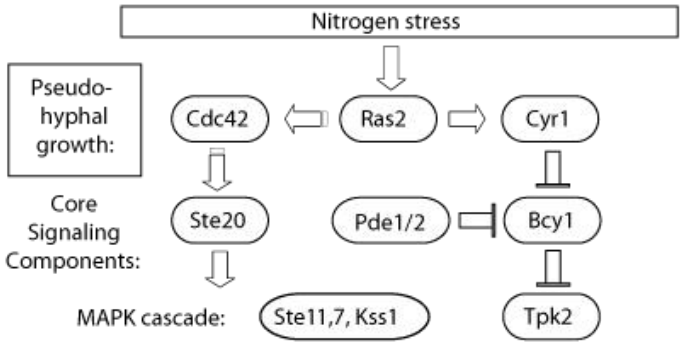
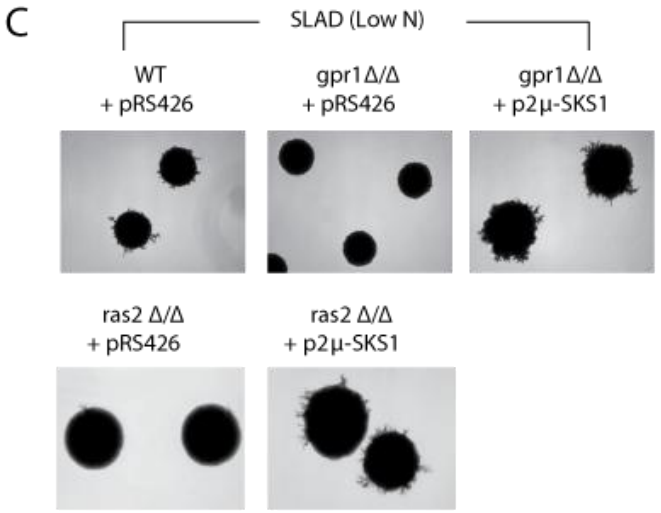


Figure 3.6. Sks1p signaling is epistatic with components of the nutrient responsive cAMP-dependent/PKA pathway. (A) Schematic of the core yeast glucose sensing components. (B) Diagram of core signaling components of yeast pseudohyphal differentiation in response to nitrogen stress. (C) Over-expression of *SKS1* from a 2μ plasmid can restore the pseudohyphal growth phenotype of both a *gpr1Δ/gpr1Δ* mutant and a *ras2Δ/ras2Δ* mutant. (D) High-copy suppression of pseudohyphal deficiency via *SKS1* was unsuccessful in diploid strains deleted for MAPK component Ste20p, PKA catalytic subunit Tpk2p, or the AMP kinase Snf1p under low nitrogen and low nitrogen, low glucose conditions.







D

Pseudohyphal Growth Phenotypes
SLAD (Low N) / SLALD (Low N, Low Glucose)

gpr1Δ/Δ	- (Low N) / - (Low N, Low D)
gpr1Δ/Δ + p2μ-SKS1	++ (Low N) / ++ (Low N, Low D)
ras2 Δ/Δ	- (Low N) / - (Low N, Low D)
ras2 Δ/Δ + p2μ-SKS1	++ (Low N) / + (Low N, Low D)
snf1 Δ/Δ	- (Low N) / - (Low N, Low D)
snf1 Δ/Δ + p2μ-SKS1	- (Low N) / - (Low N, Low D)
ste20 Δ/Δ	- (Low N) / - (Low N, Low D)
ste20 Δ/Δ + p2μ-SKS1	- (Low N) / - (Low N, Low D)
tpk2Δ/Δ	- (Low N) / - (Low N, Low D)
tpk2Δ/Δ + p2μ-SKS1	- (Low N) / - (Low N, Low D)

Figure 3.7. *SKS1* transcriptional regulation in response to nutrient deprivation. (A) Though *SKS1* mRNA levels decrease in nitrogen limiting conditions, the expression of *SKS1* nearly triples in media deficient in nitrogen and glucose. (B) Transcriptional regulation of *SKS1* involves the filamentous regulators Mss11p and Flo8p as well as the glucose-responsive regulator Rgt1p.

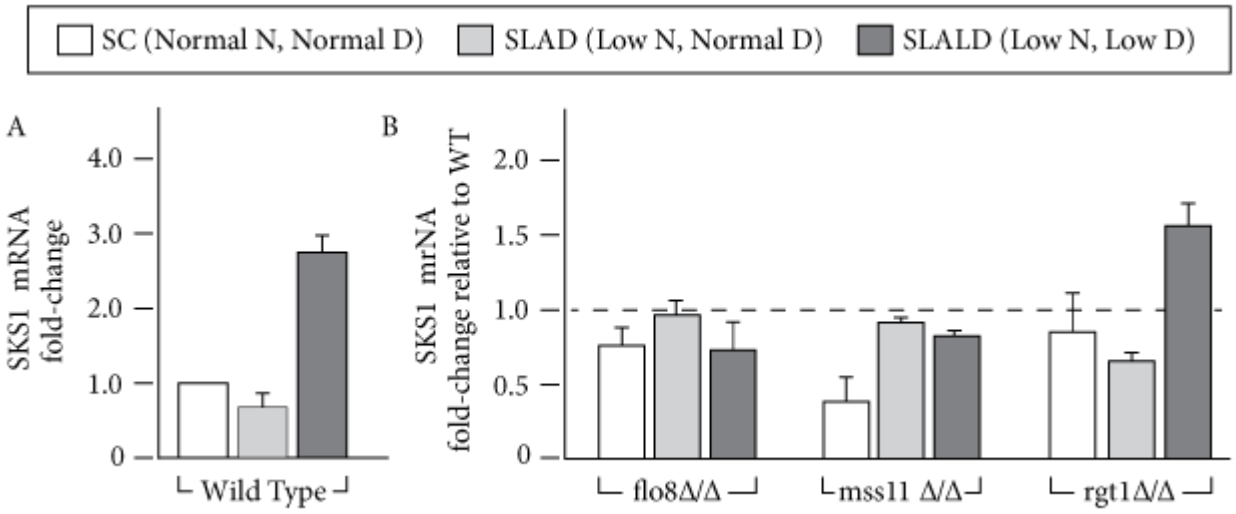


Figure 3.8. *SKS1* homologue *CaSHA3* shares a similar role in *Candida albicans*. (A) Schematic diagram demonstrating highly level of homology between *SKS1* and *CaSHA3*. (B) A *CaSHA3* heterozygous deletion strain exhibits a loss of wrinkling colony morphology on Spider media compared to wildtype strain BWP17.

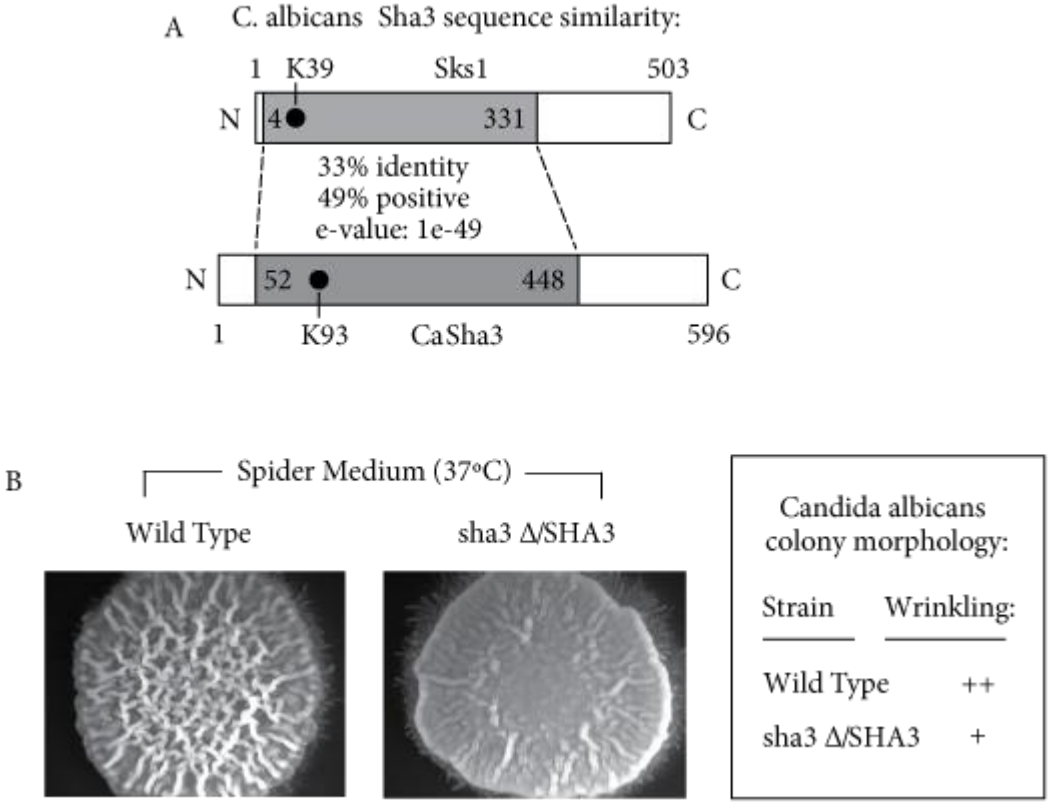


Table 3.1. Yeast strains used in this study

Strain	Genotype	Source
Y825	*ura3-52 leu2Δ0 MAT**a***	
HLY337	*ura3-52 trp1-1 MATα*	
yCK021 (825x337)	*ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK009	*Δrgt1::KanMX6/Δrg11::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK017	*Δitr1::KanMX6/Δitr1::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK036	*Δhxt1::KanMX6/Δhxt1::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK037	*Δlrg1::KanMX6/Δlrg1::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK038	*Δnpr3::KanMX6/Δnpr3::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK039	*Δpdr5::KanMX6/Δpdr5::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK040	*Δrbs1::KanMX6/Δrbs1::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK041	*Δrck2::KanMX6/Δrck2::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK042	*Δtpo4::KanMX6/Δtpo4::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK098	*Δgpr1::KanMX6/Δgpr1::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK100	*Δprb1::KanMX6/Δprb1::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK108	*Δbud6::KanMX6/Δbud6::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK110	*Δscp160::KanMX6/Δscp160::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK112	*Δsks1::KanMX6/Δsks1::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK118	*Δptr2::KanMX6/Δptr2::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK124	*Δmds3::KanMX6/Δmds3::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK151	*Δpda1::KanMX6/Δpda1::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK169	*Δmga1::KanMX6/Δmga1::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK170	*Δphd1::KanMX6/Δphd1::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK172	*Δtec1::KanMX6/Δtec1::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK189	*NPR3-S486A/NPR3-S486A ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK236	*Δmfg1::KanMX6/Δmfg1::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK237	*Δmss11::KanMX6/Δmss11::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
CS337	*Δtpk2::KanMX6/Δtpk2::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
CS343	*Δras2::KanMX6/Δras2::KanMX6 ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study

yCK263	*PDA1-S313A/PDA1-S313A ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK264	*PDA1-Y309A/PDA1-Y309A ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK274	*ITR1-S26A/ITR1-S26A ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK275	*SKS1-K39R/SKS1-K39R ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK283	*LRG1-S605A/LRG1-S605A ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK284	*PDA1-Y309A-S313A/PDA1-Y309A-S313A ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK293	*BUD6-S347A/BUD6-S347A ura3-52/ura3-52 leu2Δ0/LEU2 TRP1/trp1-1 MAT**a**/MATα*	This study
yCK300	*PDA1-3HA-KanMX6 ura3-52 leu2Δ0 MAT**a***	This study
yCK302	*PDA1-Y309A-3HA-KanMX6 ura3-52 leu2Δ0 MAT**a***	This study
yCK304	*PDA1-S313A-3HA-KanMX6 ura3-52 leu2Δ0 MAT**a***	This study
yCK306	*PDA1-Y309A-S313A-3HA-KanMX6 ura3-52 leu2Δ0 MAT**a***	This study
BWP17	*ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*	(88)
yCK307	*sha3::CdHIS1/SHA3 ura3::λimm434/ura3::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*	This Study

Table 3.2. Plasmids used in this study

Plasmid	Description	Source
pFRE-LacZ	$P_{FRE(TEC1)}$::lacZ, *URA3*, 2μ, AMP	(89)
pSKS1-vYFP	P_{SKS1}-*SKS1*, *URA3*, *Cen*, AMP	(38)
pSKS1-KD-vYFP	P_{SKS1}-*SKS1*-K39R, *URA3*, *Cen*, AMP	(38)
p426GPD	P_{GPD3}, *URA3*, 2μ, AMP	(44)
pCK020	P_{ADH1}-*SKS1*, *URA3*, 2μ, AMP	This study

Table 3.3. Previously unreported phosphosites from peptides differentially abundant in *sks1-K39R*.

Protein	Phosphosite	Localization Probability[a]	Abundance *sks1-K39R*[b]
Bul2	ASDSQDDDIRSASTT(ph)NLDR	0.78	0.50
Cdc16	NS(ph)MFGSTIPST(ph)LRKVSLQR	0.95	0.091
Dna2	HQLQEVFGQAQS(ph)R	1.0	0.14
Dse4	VS(ph)AASHSPLSVSPK	0.96	1.5
Est2	LFNVVNAS(ph)R	1.0	0.52
Irc5	DNSNSDDEEHS(ph)SKKR	0.99	0.15
Lsm4	RPYS(ph)QNR	0.99	1.5
Mds3	NS(ph)S(ph)KAVRQEGR	1.0	0.27
Nth2	ALPQLEMLGGLVACT(ph)EKSR	0.97	0.33
Nup157	MYS(ph)TPLKKR	0.99	0.30
Pal1	RGGDT(ph)QDAIK	1.0	0.31
Pat1	DLS(ph)PEEQR	1.0	2.0
Ras2	QAINVEEAFY(ph)T(ph)LARLVR	1.0	5.8
Rfc5	NIRLIMVCDS(ph)MSPIIAPIK	0.99	8.1
Sas10	S(ph)VRAVY(ph)S(ph)GGQS(ph)GVYEGEKTGIK	0.99	0.51
Ygr250c	RGNLSSSDDDDQSQT(ph)DNSSK	0.77	1.8
Ylr177w	RNTQPVLNLHPAAAPT(ph)NDAGLAVVDGK	0.87	0.43

Table 3.4. Unique phosphopeptides identified by Sks1p SILAC

Systematic Name	Standard Name	Modified Sequence	Ratio Normalized	Ratio Significance
YAL005C	SSA1	_NTIS(ph)EAGDKLEQADKDTVTK_	1.7068	0.232
YAL011W	SWC3	_GRDDDDDDDDEES(ph)DDAYDEVGNDYDEYASR_	0.72348	0.20136
YAL017W	PSK1	_ISLSPS(ph)T(ph)ESLADSK_	1.833	0.19784
YAL017W	PSK1	_ISLSPS(ph)T(ph)ESLADSK_	1.4937	0.3038
YAL019W	FUN30	_AVVEGFDETS(ph)AEPTPAPAPAPVER_	0.77098	0.23959
YAR002W	NUP60	_S(ph)RSNLSQENDNEGK_	1.7671	0.21498
YAR002W	NUP60	_SNVVVAET(ph)SPEKK_	0.81459	0.27564
YAR002W	NUP60	_SNVVVAETS(ph)PEKK_	0.70912	0.19011
YAR028W	YAR028W	_EVNSGSHSDSSSNSAEDTQS(ph)PVSAGK_	1.6164	0.26009
YAR042W	SWH1	_STLTQNDHDNDDDS(ph)TNNNNNK_	1.3531	0.36266
YAR042W	SWH1	_GSQES(ph)TNT(ph)LEEIVK_	0.74534	0.21878
YAR042W	SWH1	_KLNNQPQVETEANEES(ph)DDANSM(ox)IK_	0.73583	0.21116
YAR042W	SWH1	_FIEATKESDEDS(ph)DADEFFDAEEAASDKK_	0.53528	0.073692
YBL007C	SLA1	_NFTKS(ph)PSR_	0.77471	0.24265
YBL032W	HEK2	_KSSGEPTS(ph)PSTSSNTR_	2.1275	0.13703
YBL032W	HEK2	_S(ph)DSASFLEEKEEPQENHDNKEEQS_	1.3946	0.34422
YBL034C	STU1	_KS(ph)EGDEESDDAVDENDVKK_	0.90009	0.34713
YBL037W	APL3	_RNT(ph)IDDVNSK_	0.82997	0.28848
YBL047C	EDE1	_FVETTVENS(ph)NLNVNR_	2.498	0.087371
YBL047C	EDE1	_S(ph)QSLTSS(ph)VANNAPQSVRDDVELPETLEER_	1.4932	0.30396
YBL047C	EDE1	_YPAPGT(ph)SPSHNEGNSK_	1.3803	0.3505
YBL047C	EDE1	_ANSNEDGESVSSIQES(ph)PK_	1.0204	0.44505
YBL047C	EDE1	_SQS(ph)LTSSVANNAPQSVRDDVELPETLEER_	0.98027	0.41305
YBL047C	EDE1	_SQSLTSSVANNAPQS(ph)VRDDVELPET(ph)LEER_	0.96601	0.40149
YBL047C	EDE1	_ANSNEDGESVSS(ph)IQES(ph)PK_	0.8416	0.29821
YBL047C	EDE1	_S(ph)QSLTSS(ph)VANNAPQSVRDDVELPETLEER_	0.81517	0.27613

YBL047C	EDE1	_ANSNEDGES(ph)VSSIQES(ph)PK_	0.75008	0.2226
YBL051C	PIN4	_SLS(ph)GLDLQNQNKK_	0.98315	0.41537
YBL051C	PIN4	_S(ph)LSGLDLQNQNKK_	0.84828	0.3038
YBL051C	PIN4	_SLSHS(ph)GLDDGLEQGLNR_	0.80891	0.27092
YBL051C	PIN4	_S(ph)LSHSGLDDGLEQGLNR_	0.61787	0.12356
YBL058W	SHP1	_LGSPIPGESS(ph)PAEVPKNETPAAQEQPMPDNEPK_	1.5231	0.29271
YBL058W	SHP1	_LGS(ph)PIPGESSPAEVPKNET(ph)PAAQEQPM(ox)PDNEPK_	1.2423	0.41635
YBL058W	SHP1	_LGS(ph)PIPGESSPAEVPK_	0.99742	0.42682
YBL058W	SHP1	_KGST(ph)SPEPTK_	0.75614	0.22751
YBL058W	SHP1	_LGS(ph)TIDAADEVVEDNTSQSQR_	0.55641	0.085312
YBL066C	SEF1	_KLYPLPLYNHIS(ph)R_	2.2471	0.11831
YBL085W	BOI1	_YGNLNDS(ph)ASNIGK_	1.0745	0.48677
YBL091C	MAP2	_ADES(ph)DPVESK_	1.4138	0.33601
YBL101C	ECM21	_NSSHSLS(ph)ETDLNQSK_	1.3418	0.36785
YBL101C	ECM21	_TSGRLS(ph)VDQEPR_	1.187	0.44571
YBL101C	ECM21	_SHNSSPT(ph)NGLSQANGTVR_	1.1504	0.46611
YBR001C	NTH2	_ALPQLEMLGGLVACT(ph)EKSR_	0.70497	0.18688
YBR011C	IPP1	_AASDAIPPAS(ph)PKADAPIDK_	1.4159	0.33514
YBR054W	YRO2	_SSDS(ph)DSSIKEK_	5.3055	0.0047584
YBR054W	YRO2	_KAQEEEEDVATDS(ph)E_	4.6188	0.0090031
YBR054W	YRO2	_KM(ox)PS(ph)PASFK_	3.089	0.044045
YBR054W	YRO2	_SSDSDSS(ph)IKEK_	3.0327	0.046933
YBR054W	YRO2	_SSDSDS(ph)SIKEK_	2.8286	0.059257
YBR054W	YRO2	_KAQEEEEDVAT(ph)DSE_	2.7656	0.06374
YBR054W	YRO2	_M(ox)PSPAS(ph)FK_	2.6146	0.076071
YBR059C	AKL1	_STSYGAATIGS(ph)DEALANEK_	1.5756	0.27387
YBR059C	AKL1	_IPS(ph)QNVGQELEEEKESQSDQR_	1.5655	0.2774
YBR059C	AKL1	_ARQS(ph)LDLER_	1.3899	0.34629
YBR068C	BAP2	_KETS(ph)PDSISIR_	0.50105	0.056699

YBR072W	HSP26	_RQLANT(ph)PAK_	5.0431	0.0060419
YBR079C	RPG1	_TAGGSS(ph)PATPATPATPATPTPSSGPK_	1.0794	0.49042
YBR086C	IST2	_DANIKPVVNAAVNDNQSKVS(ph)VATEQTK_	2.3535	0.10394
YBR086C	IST2	_AVDNDTAGS(ph)AGKKPLATESTEK_	1.5849	0.27067
YBR086C	IST2	_FDEDGKS(ph)IR_	1.097	0.49717
YBR086C	IST2	_VPT(ph)VGSYGVAGATLPETIPTSK_	1.0163	0.44184
YBR086C	IST2	_AVDNDT(ph)AGS(ph)AGKKPLATESTEK_	0.67492	0.16406
YBR086C	IST2	_SSAES(ph)SNATNNNTLGTESK_	0.59017	0.10555
YBR087W	RFC5	_NIRLIM(ox)VCDSM(ox)S(ph)PIIAPIK_	877.71	2.5565E-28
YBR087W	RFC5	_NIRLIMVCDS(ph)M(ox)SPIIAPIK_	15.152	7.9986E-06
YBR108W	AIM3	_EAT(ph)GQDEVLNSITNELSHIK_	1.5903	0.26882
YBR108W	AIM3	_SQS(ph)SNSSDSSYTIDGPEANHGR_	1.5343	0.28859
YBR108W	AIM3	_VKDSSPVPS(ph)DLDEK_	1.4447	0.32318
YBR108W	AIM3	_DS(ph)SPVPSDLDEK_	1.2536	0.41056
YBR112C	CYC8	_SEVSNQSPAVVES(ph)NTNNTSQEEKPVK_	1.5964	0.26676
YBR112C	CYC8	_MREEEQTSQEKS(ph)PQENTLPR_	1.5857	0.27041
YBR112C	CYC8	_SEVSNQS(ph)PAVVESNTNNTSQEEKPVK_	1.1764	0.45155
YBR112C	CYC8	_LNS(ph)PNSNINK_	0.67927	0.1673
YBR114W	RAD16	_KSVNYNELS(ph)DDDTAVK_	0.59596	0.10922
YBR127C	VMA2	_ARDDADEDEEDPDTRS(ph)SGK_	1.8074	0.20432
YBR127C	VMA2	_VLS(ph)DKELFAINKK_	1.0849	0.49452
YBR142W	MAK5	_KAADELGIDVDS(ph)DEDDISK_	0.73406	0.20975
YBR172C	SMY2	_TS(ph)SLIDSIGIQR_	0.61939	0.12459
YBR189W	RPS9B	_KAEAS(ph)GEAAEEAEDEE_	1.7127	0.23028
YBR202W	MCM7	_FVDDGTMDT(ph)DQEDSLVST(ph)PK_	1.0073	0.43471
YBR214W	SDS24	_LSAVPM(ox)TQTPSQCLS(ph)CVHAQK_	1.0211	0.4456
YBR214W	SDS24	_SSSSTSLNSHSPLMTAMEDPPS(ph)PR_	0.96868	0.40366
YBR225W	YBR225W	_RSS(ph)DSAASSSVSK_	0.8293	0.28792
YBR279W	PAF1	_AADT(ph)PETSDAVHTEQKPEEEKETLQEE_	1.3975	0.34297

YBR279W	PAF1	_RLDDGDS(ph)DDENLDVNHIISR_	0.99006	0.42092
YCL014W	BUD3	_LGDDYS(ph)DKETAK_	1.4747	0.31117
YCL014W	BUD3	_LSPQASKVLTENS(ph)NELK_	1.3855	0.34822
YCL014W	BUD3	_AADVENLS(ph)DDDEHRQNESR_	0.58334	0.1013
YCL020W	YCL020W	_VNNDHINESTVSSQYLS(ph)DDNELSLRPATERI_	0.1372	0.000012519
YCL032W	STE50	_NKVPQISTNQSHPSAVST(ph)ANTPGPS(ph)PNEALK_	1.8687	0.18916
YCL032W	STE50	_NKVPQISTNQSHPSAVSTANTPGPS(ph)PNEALK_	0.89831	0.34565
YCL050C	APA1	_GQT(ph)PEGEDPLGKPEEELTVIPEFGGADNK_	2.0422	0.15229
YCL054W	SPB1	_KEEEGKDYIEDNDDEGVEGDS(ph)DDDEAITNLISK_	0.73097	0.20729
YCR030C	SYP1	_NVDAPVTADT(ph)PPAQTFTPSEVPPST(ph)PQQSSPPTAK_	1.3023	0.38643
YCR034W	FEN1	_NVPT(ph)PS(ph)PSPKPQHR_	0.99011	0.42097
YCR034W	FEN1	_NVPT(ph)PSPS(ph)PKPQHR_	0.92948	0.37153
YCR042C	TAF2	_SKDAQDNDEEEEEGES(ph)DEEEEEGEEERR_	1.2519	0.41143
YCR048W	ARE1	_HSVTYDNVILPQESM(ox)EVS(ph)PR_	0.78048	0.24739
YCR061W	YCR061W	_RAEGEDEGDNTS(ph)NHDTLR_	0.26331	0.0019256
YCR077C	PAT1	_DLS(ph)PEEQR_	3.846	0.019456
YCR084C	TUP1	_DVENLNTSSS(ph)PSSDLYIR_	1.3569	0.36096
YCR088W	ABP1	_SFT(ph)PSKS(ph)PAPVSK_	4.4379	0.010725
YCR088W	ABP1	_KEPVKT(ph)PS(ph)PAPAAK_	2.2061	0.12439
YCR088W	ABP1	_KEPVKTPS(ph)PAPAAK_	1.4279	0.33009
YCR088W	ABP1	_NVASGAPVQKEEPEQEEIAPSLPS(ph)RNSIPAPK_	1.361	0.35909
YCR088W	ABP1	_NVASGAPVQKEEPEQEEIAPS(ph)LPSRNSIPAPK_	1.3541	0.36221
YCR093W	CDC39	_RQT(ph)PLQSNA_	1.214	0.43117
YDL019C	OSH2	_LDGSKT(ph)PVGVHTGSALQR_	4.4096	0.011026
YDL019C	OSH2	_TAAS(ph)AGNGPTDDGTK_	2.1641	0.13098
YDL019C	OSH2	_HAPPPVPNETDNDS(ph)QYVQDEK_	1.476	0.31064
YDL019C	OSH2	_RPS(ph)NNLSVVSSEIQLNDNLTESGKR_	0.52368	0.067675
YDL022W	GPD1	_RSSSSVS(ph)LK_	1.1291	0.47826
YDL022W	GPD1	_SSS(ph)SVSLK_	1.0735	0.48596

YDL025C	RTK1	_KNTDS(ph)DQEDQIK_	0.96362	0.39955
YDL051W	LHP1	_RNS(ph)FAVIEFTPEVLDR_	0.54253	0.077585
YDL088C	ASM4	_EDDNTPAGHAGNPTNISS(ph)PIVANSPNK_	1.0135	0.43963
YDL099W	BUG1	_NKNSSATGSIGSETPDLEGTPGEES(ph)TQEETVK_	2.0093	0.15866
YDL099W	BUG1	_SENNDQNDVDEES(ph)EEKEIEQVK_	1.6839	0.2388
YDL110C	TMA17	_LEADDS(ph)DDLENIDSGDLALYK_	1.1683	0.45605
YDL122W	UBP1	_IIEHSDVENENVKDNEELQEIDNVS(ph)LDEPK_	1.1127	0.48784
YDL122W	UBP1	_FEQEFEDS(ph)EEEKEYDDAEGNYASHYNHTK_	1.0841	0.49396
YDL122W	UBP1	_IIEHS(ph)DVENENVKDNEELQEIDNVSLDEPK_	0.96695	0.40225
YDL126C	CDC48	_SVS(ph)DAELR_	0.87027	0.32221
YDL131W	LYS21	_DFHAELST(ph)PLLKPVNK_	1.4443	0.32333
YDL131W	LYS21	_DFHAELS(ph)TPLLKPVNK_	1.3457	0.36606
YDL150W	RPC53	_SEGS(ph)GSSLVQK_	1.7808	0.21128
YDL150W	RPC53	_EIQEALS(ph)EKPT(ph)REPT(ph)PSVK_	1.3081	0.38364
YDL153C	SAS10	_S(ph)VRAVY(ph)S(ph)GGQS(ph)GVYEGEKTGIK_	1.129	0.47835
YDL153C	SAS10	_GM(ox)HDNNGADLDDKDYGS(ph)EDEAVSR_	0.71316	0.19325
YDL153C	SAS10	_LNELQNS(ph)EDS(ph)DAEDGGK_	0.64787	0.14434
YDL160C	DHH1	_GSINNNFNTNNNS(ph)NTDLDRDWK_	1.4658	0.31469
YDL161W	ENT1	_GTGRS(ph)DENDDDLQR_	1.1901	0.44404
YDL161W	ENT1	_GT(ph)GRSDENDDDLQR_	1.0188	0.44376
YDL161W	ENT1	_TGTFINSQGT(ph)GYR_	0.94014	0.38032
YDL161W	ENT1	_RNQLVAASS(ph)PQQLQQQK_	0.55501	0.084519
YDL173W	PAR32	_S(ph)HQSLHATTSSPNNNAPIVVGR_	1.1285	0.47861
YDL175C	AIR2	_YFGVS(ph)DDDKDAIKEAAPK_	1.4378	0.326
YDL182W	LYS20	_NFHAEVS(ph)TPQVLSAKK_	1.3802	0.35054
YDL182W	LYS20	_NFHAEVST(ph)PQVLSAK_	1.0801	0.49093
YDL188C	PPH22	_SNDEDT(ph)DEELEDFNFKPGSSGIADHK_	0.85275	0.30754
YDL189W	RBS1	_RASVEGS(ph)PSSR_	0.47677	0.046093
YDL195W	SEC31	_VAT(ph)PLSGGVPPAPLPK_	3.5418	0.026808

YDL195W	SEC31	_VAT(ph)PLS(ph)GGVPPAPLPK_	2.4017	0.098069
YDL195W	SEC31	_VPSLVATSES(ph)PR_	1.0108	0.43743
YDL195W	SEC31	_APS(ph)SVSM(ox)VSPPPLHK_	0.98141	0.41396
YDL225W	SHS1	_KNDTYTDLASIAS(ph)GRD_	0.93992	0.38014
YDL225W	SHS1	_KNDT(ph)YTDLASIASGRD_	0.8872	0.33638
YDL225W	SHS1	_SIKTESS(ph)PK_	0.76972	0.23856
YDL225W	SHS1	_LNGSS(ph)SS(ph)INSLQQSTR_	0.72405	0.20181
YDL225W	SHS1	_FLNS(ph)PDLPER_	0.40821	0.022782
YDL226C	GCS1	_S(ph)ATPANSSNGANFQK_	3.5537	0.026469
YDL233W	MFG1	_RNS(ph)GISPR_	0.7592	0.22999
YDL240W	LRG1	_ENVFS(ph)NTKTLTLDDISR_	0.046436	7.0291E-11
YDR001C	NTH1	_RGS(ph)EDDTYSSSQGNR_	1.4176	0.33444
YDR001C	NTH1	_RGSEDDT(ph)YSSSQGNR_	1.4173	0.33453
YDR002W	YRB1	_KEGDDAPES(ph)PDIHFEPVVHLEK_	1.4861	0.30672
YDR011W	SNQ2	_VQDLINDLSKQET(ph)K_	0.91951	0.36328
YDR011W	SNQ2	_STQDS(ph)SHNAVAR_	0.28951	0.0034943
YDR017C	KCS1	_RNS(ph)NTTTM(ox)GNHNAR_	0.68065	0.16833
YDR028C	REG1	_DSEGNAQS(ph)EEEHDLER_	2.2904	0.11221
YDR028C	REG1	_GYGS(ph)DDENSK_	1.3876	0.3473
YDR028C	REG1	_KS(ph)DVKPQENGNDSS_	1.194	0.44188
YDR028C	REG1	_SDSGVHS(ph)PITDNSSVASSTTSR_	1.161	0.46008
YDR028C	REG1	_SDVKPQENGNDSS(ph)_	1.0504	0.46842
YDR028C	REG1	_KSDVKPQENGNDS(ph)S_	0.91831	0.36228
YDR033W	MRH1	_APVAS(ph)PRPAATPNLS(ph)K_	3.8956	0.018483
YDR033W	MRH1	_APVAS(ph)PR_	2.466	0.090779
YDR033W	MRH1	_PAAT(ph)PNLSK_	1.7957	0.20737
YDR043C	NRG1	_NTLS(ph)DEEDLEQR_	0.90375	0.35018
YDR060W	MAK21	_ASNFDS(ph)DDEMDENEIWSALVK_	0.80618	0.26865
YDR060W	MAK21	_AS(ph)NFDSDDEMDENEIWSALVK_	0.58338	0.10133

YDR063W	AIM7	_LIEVSSGLEDDS(ph)DVEELR_	1.0198	0.44453
YDR074W	TPS2	_RSAS(ph)YTGAKV_	1.7262	0.22636
YDR119W	VBA4	_IDDS(ph)PQDEVNS(ph)IK_	1.7471	0.22048
YDR119W	VBA4	_IDDSPQDEVNS(ph)IK_	0.43961	0.03224
YDR122W	KIN1	_EAYAAQKFEGS(ph)DDDENHPLPPLNVAK_	1.0525	0.46998
YDR135C	YCF1	_RASDAT(ph)LGSIDFGDDENIAKR_	1.1319	0.47669
YDR135C	YCF1	_RAS(ph)DATLGSIDFGDDENIAKR_	1.0551	0.47195
YDR135C	YCF1	_RAS(ph)DATLGS(ph)IDFGDDENIAKR_	0.96714	0.40241
YDR135C	YCF1	_LNDLDFGNSDAIS(ph)LR_	0.9332	0.3746
YDR141C	DOP1	_IKM(ox)EAEENPS(ph)ETETNDSHLDR_	1.4051	0.33972
YDR153C	ENT5	_IDDLLDWDGPKS(ph)DTDTTAAAQTSLPFAEKK_	0.71057	0.19123
YDR155C	CPR1	_KVESLGS(ph)PSGATK_	1.5041	0.29984
YDR158W	HOM2	_NRDS(ph)GYGVSVGR_	0.64559	0.14272
YDR159W	SAC3	_LNQNSSVKPQINT(ph)SPK_	0.5979	0.11046
YDR168W	CDC37	_WDKIELSDDS(ph)DVEVHPNVDKK_	4.1447	0.014341
YDR170C	SEC7	_ETSNDAT(ph)NGM(ox)KTPEETEDTNDKR_	2.7401	0.065658
YDR170C	SEC7	_ETSNDATNGMKT(ph)PEETEDTNDKR_	2.5984	0.077541
YDR170C	SEC7	_RNES(ph)NEDIR_	1.4149	0.33556
YDR171W	HSP42	_KS(ph)S(ph)SFAHLQAPSPIPDPLQVSKPETR_	3.9079	0.018249
YDR171W	HSP42	_KS(ph)SSFAHLQAPSPIPDPLQVSKPETR_	2.3642	0.1026
YDR171W	HSP42	_KS(ph)SS(ph)FAHLQAPSPIPDPLQVSKPETR_	2.1683	0.1303
YDR171W	HSP42	_SSSFAHLQAPS(ph)PIPDPLQVSKPETR_	1.5907	0.26869
YDR173C	ARG82	_LSDS(ph)TDNLDSIPVKSEK_	2.8364	0.058726
YDR173C	ARG82	_LS(ph)DSTDNLDSIPVK_	2.7145	0.067652
YDR186C	YDR186C	_NLINKS(ph)FEELR_	1.3865	0.34777
YDR186C	YDR186C	_NSSS(ph)LLNFQNSVLTSNKDK_	1.1749	0.45236
YDR186C	YDR186C	_NNS(ph)NSNLLFEK_	0.85928	0.31301
YDR186C	YDR186C	_S(ph)GSSTDVLSSGIDSMAK_	0.75549	0.22697
YDR207C	UME6	_GGS(ph)PSNDSQVQHNVHDDQCAVGVAPR_	1.3009	0.38714

YDR229W	IVY1	_PDNNTEQLQGS(ph)PSSDQR_	1.564	0.27792
YDR229W	IVY1	_RPVASSAGS(ph)ENNDHLDDMNHLR_	1.3962	0.34356
YDR229W	IVY1	_RPVAS(ph)SAGSENNDHLDDMNHLR_	1.3269	0.37477
YDR229W	IVY1	_PDNNTEQLQGS(ph)PSS(ph)DQR_	1.3138	0.38095
YDR264C	AKR1	_SGNEEES(ph)GNEQVNHNDEAEEDPLLTR_	1.3776	0.35168
YDR266C	HEL2	_KGSS(ph)STSLNNSK_	0.6022	0.11323
YDR293C	SSD1	_SST(ph)INNDSDSLSSPTK_	3.0183	0.047701
YDR293C	SSD1	_NQSQQPQQQLS(ph)PFR_	2.16	0.13164
YDR293C	SSD1	_SSTINNDSDS(ph)LSSPTK_	1.7071	0.23188
YDR293C	SSD1	_SSTINNDSDSLSS(ph)PTK_	1.2375	0.41885
YDR299W	BFR2	_DSVDDNENS(ph)DDGLDIPK_	0.65385	0.14862
YDR299W	BFR2	_NGES(ph)DLS(ph)DYGNSNTEETK_	0.50162	0.056962
YDR303C	RSC3	_KLS(ph)EDGVTDGDGKPIPESER_	0.89178	0.3402
YDR310C	SUM1	_TAGDDGALDQTENTSIS(ph)PK_	0.5244	0.068037
YDR320C	SWA2	_TDKSPNDSLHSISAPPLIPS(ph)PK_	0.67642	0.16517
YDR330W	UBX5	_VNDMFDEGRPES(ph)IFNQR_	1.4589	0.31745
YDR333C	RQC1	_DKGS(ph)DDDDDDEEFDKIIQQFK_	1.0171	0.44242
YDR345C	HXT3	_SSENSNADLPSNS(ph)SQVM(ox)NMPEEK_	1.7717	0.21374
YDR345C	HXT3	_SSENSNADLPS(ph)NSS(ph)QVM(ox)NMPEEK_	1.7015	0.23356
YDR345C	HXT3	_S(ph)SENSNADLPSNSSQVMNMPEEK_	1.6322	0.25496
YDR348C	PAL1	_TSSQLS(ph)FPNIPEDEPQR_	1.0668	0.48091
YDR348C	PAL1	_RGGDT(ph)QDAIK_	0.60876	0.11751
YDR356W	SPC110	_NTTQTQVVS(ph)PTKVPNANNGDENEGPVK_	0.87489	0.32609
YDR356W	SPC110	_DELNELET(ph)KFSENGS(ph)QSS(ph)AK_	0.5773	0.097608
YDR365C	ESF1	_EAENDEDS(ph)EVNAK_	0.88586	0.33526
YDR365W-B	YDR365W-B	_EVHTNQDPLDVS(ph)ASKTEECEK_	3.3471	0.033091
YDR365W-B	YDR365W-B	_AHNVSTSNNSPSTDNDS(ph)ISK_	2.1174	0.13875
YDR365W-B	YDR365W-B	_NLS(ph)DEKNDSR_	2.1069	0.14055
YDR365W-B	YDR365W-B	_EVHTNQDPLDVSAS(ph)KTEECEKASTK_	1.7881	0.20935

YDR365W-B	YDR365W-B	_EVHTNQDPLDVSASKTEECEKAS(ph)TK_	1.666	0.24428
YDR365W-B	YDR365W-B	_AHNVST(ph)SNNSPSTDNDSISK_	1.2416	0.41674
YDR365W-B	YDR365W-B	_TVPQIS(ph)DQETEKR_	0.90593	0.352
YDR365W-B	YDR365W-B	_AVS(ph)PTDSTPPSTHTEDSK_	0.6223	0.12655
YDR365W-B	YDR365W-B	_HSDSYS(ph)ENETNHTNVPISSTGGTNNK_	0.61975	0.12483
YDR365W-B	YDR365W-B	_AVSPT(ph)DSTPPSTHTEDSKR_	0.6007	0.11226
YDR365W-B	YDR365W-B	_SPS(ph)IDASPPENNSSHNIVPIK_	0.39216	0.018722
YDR365W-B	YDR365W-B	_AHNVSTSNNSPST(ph)DNDSISK_	0.057369	1.0772E-09
YDR372C	VPS74	_ADSGDTSS(ph)IHSSANNTKGDK_	1.678	0.2406
YDR372C	VPS74	_ADSGDTSSIHSS(ph)ANNTK_	1.5294	0.29039
YDR372C	VPS74	_ADSGDTSSIHS(ph)SANNTK_	1.2325	0.42143
YDR372C	VPS74	_ADS(ph)GDTSSIHSSANNTKGDK_	0.78344	0.24983
YDR379W	RGA2	_NSS(ph)KEDFPIKLPER_	2.1169	0.13883
YDR379W	RGA2	_VHDELPS(ph)PGKVPLS(ph)PSPK_	0.65588	0.15009
YDR382W	RPP2B	_EEEAKEES(ph)DDDM(ox)GFGLFD_	0.85344	0.30812
YDR407C	TRS120	_LNFESSSTGIS(ph)PVDSNSK_	0.47857	0.046835
YDR422C	SIP1	_VNSS(ph)NSM(ox)YTAER_	0.66921	0.15982
YDR428C	BNA7	_AIS(ph)PDITLFNK_	1.7631	0.21607
YDR436W	PPZ2	_GTSPIPNLNIDKPS(ph)PSASSASKR_	0.98535	0.41714
YDR436W	PPZ2	_INTSS(ph)SADR_	0.96765	0.40283
YDR439W	LRS4	_GGNDPDS(ph)PTSQRR_	0.77322	0.24143
YDR454C	GUK1	_RLS(ph)AAQAELAYAETGAHDK_	0.98628	0.41789
YDR458C	HEH2	_ANKPPESPPQS(ph)K_	0.94473	0.3841
YDR458C	HEH2	_ANKPPES(ph)PPQSK_	0.92494	0.36778
YDR460W	TFB3	_LKDAVPFT(ph)PFNGDR_	1.2065	0.43517
YDR496C	PUF6	_ISIDS(ph)S(ph)DEESELSK_	0.53832	0.075314
YDR497C	ITR1	_TSQSNVGDAVGNADS(ph)VEFNSEHDSPSKR_	0.36844	0.013636
YDR505C	PSP1	_KGS(ph)FTTELSCR_	0.59604	0.10927
YDR507C	GIN4	_KQNS(ph)QEGDQAHPK_	1.1491	0.46681

YDR507C	GIN4	_LSTIVNQSS(ph)PTPASR_	0.60694	0.11631
YDR507C	GIN4	_LDPGIMFSSPTEEVS(ph)PVEPK_	0.54787	0.080514
YDR517W	GRH1	_ESDDLDDVS(ph)LNHDER_	1.513	0.29645
YEL013W	VAC8	_DSSDEAS(ph)VSPIADNER_	1.076	0.48788
YEL015W	EDC3	_LGQM(ox)IISQS(ph)R_	0.89012	0.33881
YEL015W	EDC3	_LGQMIISQS(ph)RSNS(ph)TSLPAANK_	0.666	0.15746
YEL017W	GTT3	_EDDDEKPQSGDET(ph)SATKPLSSR_	2.3661	0.10238
YEL022W	GEA2	_VNNEEAIS(ph)EDDGIEEEHIHSEK_	1.0892	0.49776
YEL037C	RAD23	_TESAS(ph)TPGFVVGTER_	1.2632	0.4057
YEL043W	YEL043W	_S(ph)SGSIQLPLSNNMSR_	547.32	1.0027E-24
YEL046C	GLY1	_S(ph)ESTEVDVDGNAIR_	0.66118	0.15393
YEL046C	GLY1	_SES(ph)TEVDVDGNAIR_	0.49813	0.055359
YEL046C	GLY1	_SEST(ph)EVDVDGNAIR_	0.39691	0.019872
YEL060C	PRB1	_DDDEEPS(ph)DSEDKEHGK_	0.10926	1.4556E-06
YER024W	YAT2	_KTSSSS(ph)QVNLNR_	0.20703	0.0003643
YER042W	MXR1	_VGYANGEESKKDS(ph)PSSVSYK_	1.2799	0.39738
YER052C	HOM3	_KGESTPPHPPENLS(ph)SSFYEK_	4.4636	0.01046
YER052C	HOM3	_KGES(ph)TPPHPPENLSSS(ph)FYEKR_	4.0158	0.01634
YER052C	HOM3	_KGEST(ph)PPHPPENLSSSFYEKR_	1.3061	0.38464
YER052C	HOM3	_KGES(ph)TPPHPPENLSSSFYEK_	1.1865	0.44601
YER059W	PCL6	_HES(ph)PSNESSLDK_	0.99683	0.42635
YER059W	PCL6	_M(ox)SACNSNENNENDDS(ph)DDENTGVQR_	0.90678	0.3527
YER070W	RNR1	_EAS(ph)PAPTGSHSLTK_	1.0291	0.45184
YER074W-A	YOS1	_SNDET(ph)PVFGQDQNTTK_	1.4418	0.32435
YER079W	YER079W	_SM(ox)S(ph)PSNIASGEDR_	1.9675	0.16715
YER079W	YER079W	_PDSSHS(ph)ISSK_	1.2194	0.42831
YER079W	YER079W	_TNS(ph)GCSITSGASMIATK_	0.82019	0.28031
YER100W	UBC6	_KLDEGDAANT(ph)GDETEDPFTK_	0.30366	0.0046548
YER105C	NUP157	_M(ox)YS(ph)TPLKKR_	0.61335	0.12055

YER112W	LSM4	_RPYS(ph)QNR_	2.5637	0.080798
YER114C	BOI2	_KT(ph)SQSLEDLPSQQNFIPTPR_	1.5877	0.26971
YER114C	BOI2	_DKDVSDTLSPDFDSKGS(ph)ATGR_	1.3274	0.37451
YER114C	BOI2	_NSS(ph)PIVDKK_	0.81186	0.27337
YER114C	BOI2	_DKDVSDT(ph)LSPDFDSKGSATGR_	0.54034	0.076396
YER122C	GLO3	_AIS(ph)SDQLFGR_	1.1105	0.48914
YER129W	SAK1	_SRS(ph)LTVAELNEEK_	1.4384	0.32578
YER129W	SAK1	_SRSLT(ph)VAELNEEK_	1.1365	0.47399
YER149C	PEA2	_NT(ph)SSPPIS(ph)PNAAAIQEEDSSK_	0.65007	0.14591
YER164W	CHD1	_AAAHQQNYFNDS(ph)DDEDDEDNIKQSR_	0.74142	0.21563
YER169W	RPH1	_KES(ph)PVETSK_	2.2042	0.12469
YER169W	RPH1	_NDDLDKEQGSS(ph)PLNSK_	1.2633	0.40569
YER169W	RPH1	_NDDLDKEQGS(ph)SPLNSK_	0.90992	0.35531
YER176W	ECM32	_KENSTIQSSS(ph)SSNLR_	0.96871	0.40368
YER178W	PDA1	_YGGHS(ph)MSDPGTTYR_	0.50333	0.05776
YER178W	PDA1	_Y(ph)GGHSMSDPGTTYR_	0.46437	0.041146
YFL004W	VTC2	_LMGVDS(ph)EEEEIELPPGVK_	1.1486	0.46711
YFL005W	SEC4	_EGNISINSGS(ph)GNSSK_	1.6025	0.26471
YFL005W	SEC4	_EGNIS(ph)INSGSGNSSK_	1.3537	0.36239
YFL023W	BUD27	_ILENIS(ph)DDDYDDDDDGNKK_	0.89101	0.33956
YFL033C	RIM15	_KGSIIGDNQQTTANSSDS(ph)PTMTK_	1.0832	0.49328
YFL038C	YPT1	_ESM(ox)S(ph)QQNLNETTQK_	2.0548	0.14993
YFR003C	YPI1	_SYS(ph)PNAYEIQPDYSEYRR_	1.2535	0.41065
YFR003C	YPI1	_S(ph)YSPNAYEIQPDYSEYR_	1.0354	0.45675
YFR010W	UBP6	_KFDPSSSENVM(ox)T(ph)PR_	1.0816	0.49208
YFR017C	IGD1	_S(ph)TNYM(ox)DALNSR_	2.442	0.093429
YFR024C-A	LSB3	_ISSAST(ph)PQTSQGR_	1.2943	0.3903
YFR024C-A	LSB3	_LAPTNS(ph)GGSGGK_	1.2881	0.39336
YFR024C-A	LSB3	_LAPTNS(ph)GGSGGKLDDPSGASSYYASHR_	0.88128	0.33143

YFR024C-A	LSB3	_GSFDDDEDDYYDDDDYYNDIPSSFS(ph)STDASSTRPNTR_	0.73115	0.20743
YFR024C-A	LSB3	_ISSAS(ph)T(ph)PQTSQGR_	0.68526	0.1718
YFR024C-A	LSB3	_GSFDDDEDDYYDDDDYYNDIPS(ph)SFSSTDASSTRPNTR_	0.56876	0.092488
YFR024C-A	LSB3	_FTAPTSPS(ph)TSSPK_	0.34789	0.010057
YFR028C	CDC14	_KVVIESNNS(ph)DDESMQDTNGTSNHYPK_	0.74038	0.2148
YFR038W	IRC5	_DNSNSDDEEHS(ph)SKKR_	0.30977	0.005234
YFR053C	HXK1	_TKYDVAVDEQS(ph)PRPGQQAFEK_	2.748	0.065061
YFR053C	HXK1	_KGS(ph)MADVPK_	1.0008	0.42949
YGL008C	PMA1	_VS(ph)TQHEKET_	4.5806	0.0093397
YGL008C	PMA1	_SVEDFM(ox)AAM(ox)QRVS(ph)TQHEKET(ph)_	3.2061	0.03865
YGL008C	PMA1	_VST(ph)QHEKET_	1.7437	0.22141
YGL009C	LEU1	_VEVTSEDEKELES(ph)AAYDHAEPVQPEDAPQDIANDELKDIPVK_	0.22924	0.00075805
YGL009C	LEU1	_DQDQSSPKVEVT(ph)SEDEK_	0.20325	0.00031766
YGL009C	LEU1	_EFEYKDQDQSS(ph)PK_	0.19929	0.00027416
YGL009C	LEU1	_EFEYKDQDQS(ph)SPKVEVTS(ph)EDEK_	0.16989	0.000078317
YGL009C	LEU1	_VEVTS(ph)EDEK_	0.1455	0.000021099
YGL014W	PUF4	_AKKPS(ph)VGANNTAK_	0.56879	0.092503
YGL023C	PIB2	_KNS(ph)AEENVR_	0.91384	0.35858
YGL030W	RPL30	_APVKS(ph)QESINQK_	0.88432	0.33397
YGL049C	TIF4632	_FNNHNQSNS(ph)NR_	1.9505	0.17074
YGL049C	TIF4632	_FNNHNQS(ph)NSNR_	1.9354	0.17398
YGL049C	TIF4632	_TVNKS(ph)DDETINSVITTEENTVK_	1.4143	0.33581
YGL049C	TIF4632	_SNS(ph)RFNNHNQSNSNR_	1.1794	0.44991
YGL077C	HNM1	_VEEEIKPLDDM(ox)DS(ph)K_	0.61173	0.11947
YGL077C	HNM1	_EFSVAAS(ph)DVELENEHVPWGKK_	0.3942	0.01921
YGL083W	SCY1	_KDGS(ph)SDIPR_	1.2249	0.42543
YGL092W	NUP145	_AYEPDLSDADFEGIEAS(ph)PK_	1.3223	0.37692
YGL092W	NUP145	_TDGTFGTLSGKDDS(ph)IVEEK_	1.0651	0.47965

YGL092W	NUP145	_AYEPDLS(ph)DADFEGIEASPK_	0.87441	0.32568
YGL120C	PRP43	_RFS(ph)SEHPDPVETSIPEQAAEIAEELSK_	0.33794	0.0085821
YGL139W	FLC3	_NENAS(ph)TDALRVEAPK_	0.67132	0.16138
YGL171W	ROK1	_TTENDS(ph)PNKEEK_	0.71076	0.19138
YGL173C	XRN1	_KGEIKPSSGTNSTECQS(ph)PK_	0.76251	0.23268
YGL178W	MPT5	_NAS(ph)ISNM(ox)PAM(ox)NTAR_	1.5819	0.2717
YGL180W	ATG1	_SMDSNAIAEEQDS(ph)DDAEEEDETLKK_	1.2789	0.3979
YGL197W	MDS3	_AQS(ph)SSTQESSGSANGEK_	1.1841	0.4473
YGL197W	MDS3	_NS(ph)S(ph)KAVRQEGR_	0.4551	0.037659
YGL208W	SIP2	_KVTELS(ph)LNK_	1.7882	0.20933
YGL208W	SIP2	_KVT(ph)ELSLNK_	1.4106	0.3374
YGL227W	VID30	_SRS(ph)AINIETESR_	0.88939	0.33821
YGL232W	TAN1	_FQELYGDIKEGEDES(ph)ENDEKK_	0.99921	0.42825
YGL244W	RTF1	_RIEVGSVEDDDEEDDYNPYSVGNADYGS(ph)EEEEEANPFPLEGK_	1.0054	0.43314
YGL253W	HXK2	_KGS(ph)M(ox)ADVPKELMQQIENFEK_	0.91604	0.3604
YGR008C	STF2	_RGS(ph)NLQSHEQK_	1.0263	0.4497
YGR048W	UFD1	_S(ph)KAPKSPEVIEID_	0.91957	0.36333
YGR048W	UFD1	_APKS(ph)PEVIEID_	0.86979	0.32181
YGR054W	YGR054W	_SSETS(ph)PDSTPAPSAPASTNAPTNNKETSPEEKK_	4.7413	0.00801
YGR054W	YGR054W	_SSETS(ph)PDSTPAPSAPASTNAPTNNKETSPEEK_	1.4658	0.31469
YGR054W	YGR054W	_ANKKS(ph)S(ph)ETSPDSTPAPSAPASTNAPTNNK_	1.3887	0.34681
YGR054W	YGR054W	_S(ph)SETSPDSTPAPSAPASTNAPTNNK_	1.2543	0.41025
YGR082W	TOM20	_GVVGSKAES(ph)DAVAEANDIDD_	0.27562	0.0025765
YGR086C	PIL1	_ALLELLDDSPVT(ph)PGETRPAYDGYEASK_	4.0058	0.016508
YGR086C	PIL1	_YKDPQS(ph)PK_	1.534	0.28868
YGR093W	YGR093W	_RPLETET(ph)ENSFDGDKQVLANR_	1.894	0.18325
YGR097W	ASK10	_GNNS(ph)AQNLTTSSSTASR_	1.0226	0.44673
YGR100W	MDR1	_AVDLAEEVNLIDLS(ph)DDEGEEKR_	1.3386	0.36933

YGR103W	NOP7	_SALNADEANTDET(ph)EKEEEQEKK_	0.9029	0.34947
YGR116W	SPT6	_VGDEGNAAES(ph)ESDNVAASR_	0.90124	0.34809
YGR116W	SPT6	_VGDEGNAAES(ph)ES(ph)DNVAASR_	0.88442	0.33406
YGR116W	SPT6	_EREEDDRLS(ph)EDDLDLLMENAGVER_	0.68556	0.17203
YGR125W	YGR125W	_TIEENIDEEYS(ph)DEYSR_	1.8142	0.20259
YGR128C	UTP8	_ALEDTTDTSNDHLS(ph)ESDIDNK_	1.1124	0.48804
YGR145W	ENP2	_ALTAAEES(ph)DEER_	0.71738	0.19656
YGR148C	RPL24B	_KVEVDS(ph)FSGAK_	0.55744	0.085903
YGR152C	RSR1	_VKQST(ph)PVNEK_	2.1181	0.13863
YGR162W	TIF4631	_LKETSDSTSTSTPT(ph)PTPSTNDSK_	1.7666	0.21511
YGR162W	TIF4631	_LKETSDSTSTST(ph)PTPTPSTNDSK_	1.5059	0.29915
YGR162W	TIF4631	_STVS(ph)PQPESK_	0.71964	0.19833
YGR162W	TIF4631	_APPPKEEPAAPTSTATNM(ox)FSALM(ox)GES(ph)DDEE_	0.67048	0.16076
YGR167W	CLC1	_EAEILGDEFKTEQDDILETEAS(ph)PAKDDDEIR_	2.7758	0.062988
YGR178C	PBP1	_S(ph)GSNISQGQSSTGHTTR_	1.7169	0.22905
YGR178C	PBP1	_VADSGVSDS(ph)VDDLAK_	1.6328	0.25475
YGR178C	PBP1	_TVS(ph)PTTQISAGK_	1.4426	0.32403
YGR180C	RNR4	_SAT(ph)PSKEINFDDDF_	2.0237	0.15584
YGR186W	TFG1	_DGSQT(ph)PTVDSVTK_	1.0866	0.49578
YGR186W	TFG1	_DEEAPS(ph)ENEEDELFGEKK_	0.98019	0.41298
YGR186W	TFG1	_AVDSSNNASNTVPS(ph)PIKQEEGLNSTVAER_	0.61993	0.12495
YGR192C	TDH3	_TAS(ph)GNIIPSSTGAAK_	1.2044	0.43631
YGR192C	TDH3	_TASGNIIPS(ph)STGAAK_	1.1481	0.46741
YGR192C	TDH3	_IVSNASCT(ph)TNCLAPLAK_	0.93146	0.37317
YGR196C	FYV8	_SAS(ph)FKSENR_	0.83202	0.29019
YGR196C	FYV8	_GYLADILPAEKEENLQQEDDGEVES(ph)SGALEKK_	0.0042367	8.31E-30
YGR202C	PCT1	_EAS(ph)PATEFANEFTGENSTAK_	1.2331	0.42113
YGR202C	PCT1	_NQDENKDTQLT(ph)PR_	0.77828	0.24558
YGR229C	SMI1	_SQQGLSHVTSTGSSS(ph)SM(ox)ER_	1.1052	0.4923

YGR240C	PFK1	_KNEAS(ph)PNTDAK_	3.5453	0.026707
YGR240C	PFK1	_KNEASPNT(ph)DAK_	3.0534	0.045847
YGR240C	PFK1	_ASS(ph)DAS(ph)DLLR_	1.7948	0.20759
YGR240C	PFK1	_ASS(ph)DASDLLR_	1.4543	0.3193
YGR240C	PFK1	_DAFLEATS(ph)EDEIISR_	1.1984	0.43953
YGR250C	YGR250C	_YFDS(ph)VRST(ph)PVAEK_	3.8395	0.019587
YGR250C	YGR250C	_RGNLSSSDDDDQSQT(ph)DNSSK_	3.4146	0.030746
YGR250C	YGR250C	_YFDS(ph)VRS(ph)TPVAEK_	3.1763	0.039952
YGR250C	YGR250C	_GNLSSS(ph)DDDDQSQTDNSSK_	2.5167	0.085435
YGR250C	YGR250C	_MDLFYPQRES(ph)FSEGR_	1.1211	0.48295
YGR253C	PUP2	_ATS(ph)PLLESDSIEK_	1.6497	0.24935
YGR253C	PUP2	_AT(ph)SPLLESDSIEK_	1.5339	0.28872
YGR253C	PUP2	_EKEAAES(ph)PEEADVEM(ox)S_	1.2787	0.39799
YGR261C	APL6	_LTGINDGDSNS(ph)ISGK_	1.0425	0.46232
YGR261C	APL6	_IAS(ph)ALDTAK_	0.80011	0.2636
YGR267C	FOL2	_HET(ph)PLNIRPT(ph)SPYTLNPPVER_	1.8523	0.19311
YGR267C	FOL2	_HETPLNIRPTS(ph)PYTLNPPVER_	0.43351	0.030242
YGR270W	YTA7	_ELQEDS(ph)PIREK_	0.92629	0.3689
YGR270W	YTA7	_HPET(ph)PPPVRR_	0.87263	0.32419
YGR270W	YTA7	_RGS(ph)DVEDASNAK_	0.79563	0.2599
YGR280C	PXR1	_SSES(ph)ASNIPDAVNTR_	0.70434	0.1864
YGR281W	YOR1	_TITVGDAVSET(ph)ELENK_	0.44918	0.035529
YGR285C	ZUO1	_NHTWS(ph)EFER_	0.2915	0.0036431
YHL007C	STE20	_RAT(ph)PVS(ph)TPVISKPS(ph)M(ox)TTTPR_	1.7975	0.20688
YHL007C	STE20	_RAT(ph)PVS(ph)TPVISKPSMTTTPR_	1.6117	0.26165
YHL007C	STE20	_HQQPVASSTVNSNKSS(ph)TDIR_	1.3613	0.35896
YHL007C	STE20	_HQQPVASSTVNS(ph)NK_	0.93381	0.37511
YHL007C	STE20	_SLS(ph)KELNEK_	0.9244	0.36733
YHL007C	STE20	_LSLTDS(ph)TETIENNATVK_	0.88475	0.33433

YHL007C	STE20	_RAT(ph)PVSTPVISKPSM(ox)TTT(ph)PR_	0.72248	0.20057
YHL007C	STE20	_LSLTDS(ph)TET(ph)IENNATVK_	0.70624	0.18786
YHL007C	STE20	_SPVM(ox)NSAANVS(ph)PLK_	0.69109	0.17623
YHL023C	NPR3	_KVNS(ph)GEADTVYDSNIGHEASTDTPNLR_	0.42824	0.028581
YHL034C	SBP1	_FPT(ph)KIDFDNIKENYDTK_	3.7514	0.021472
YHL034C	SBP1	_ELTVDVAVIRPENDEEEIEQET(ph)GSEEKQE_	2.226	0.12139
YHR005C	GPA1	_LLLLGAGESGKS(ph)TVLK_	0.90071	0.34765
YHR016C	YSC84	_GYGDFDS(ph)EDEDYDYGRSPNR_	1.3417	0.36787
YHR016C	YSC84	_RGMS(ph)LGSR_	1.0745	0.48674
YHR027C	RPN1	_QQTIDEQSQISPEKQT(ph)PNKK_	2.0917	0.14323
YHR030C	SLT2	_GYSENPVENSQFLT(ph)EYVATR_	0.72948	0.2061
YHR052W	CIC1	_KS(ph)TPVS(ph)TPSKEK_	1.9898	0.16256
YHR052W	CIC1	_KST(ph)PVST(ph)PSKEK_	1.4957	0.303
YHR052W	CIC1	_SSSELEKESS(ph)ESEAVK_	1.0307	0.45314
YHR052W	CIC1	_KS(ph)TPVS(ph)TPSKEK_	0.96817	0.40325
YHR052W	CIC1	_SSSELEKES(ph)SESEAVKK_	0.91253	0.35749
YHR056C	RSC30	_SDS(ph)PDVPSMDQIR_	1.23	0.42273
YHR056C	RSC30	_S(ph)DSPDVPSM(ox)DQIR_	1.1148	0.48661
YHR064C	SSZ1	_EENAEEDDESEWS(ph)DDEPEVVR_	1.1216	0.48265
YHR064C	SSZ1	_EENAEEDDES(ph)EWSDDEPEVVR_	0.91809	0.3621
YHR094C	HXT1	_NESFHDNLSES(ph)QVQPAVAPPNTGK_	0.37509	0.014954
YHR094C	HXT1	_NESFHDNLS(ph)ESQVQPAVAPPNTGK_	0.36029	0.012128
YHR094C	HXT1	_NES(ph)FHDNLS(ph)ESQVQPAVAPPNTGK_	0.35407	0.011056
YHR099W	TRA1	_KEDINDS(ph)PDVEMTESDKVVK_	1.3083	0.38358
YHR114W	BZZ1	_LGSAPNNAGEDS(ph)DNNSIR_	1.2411	0.41697
YHR114W	BZZ1	_LGSAPNNAGEDSDNNS(ph)IR_	0.99734	0.42675
YHR117W	TOM71	_RQS(ph)EAFAGQNEDEADLKDDGSVVSGSNK_	0.87823	0.32887
YHR146W	CRP1	_RES(ph)TEGVLDGSK_	0.85727	0.31133
YHR146W	CRP1	_KQTAS(ph)PLSSSTEEPK_	0.8282	0.287

YHR146W	CRP1	_EET(ph)PLAEPTNVSK_	0.3048	0.0047592
YHR158C	KEL1	_NNS(ph)PILETLPSNEIK_	1.0468	0.46563
YHR158C	KEL1	_NVS(ph)PM(ox)GDVPTDTKNEEASVPINR_	0.96919	0.40407
YHR159W	TDA11	_SVS(ph)PQDIQER_	0.97797	0.41119
YHR164C	DNA2	_HQLQEVFGQAQS(ph)R_	0.31357	0.0056188
YIL021W	RPB3	_KVAS(ph)ILLALT(ph)QM(ox)DQDKVNFAS(ph)GDNNT(ph)AS(ph)NMLGSNEDVMMTGAEQDPYSNASQM(ox)GNTGSGGYDNAW_	3.2596	0.03643
YIL026C	IRR1	_SQVIEEDYDDEQNTS(ph)AQHVESDKITAK_	0.80873	0.27077
YIL033C	BCY1	_RTS(ph)VSGETLQPNNFDDWTPDHYKEK_	2.347	0.10477
YIL033C	BCY1	_TST(ph)PPLPMHFNAQR_	1.3169	0.37946
YIL036W	CST6	_FGSDT(ph)DDDDIDLKPVEGGKDPDNQSLPNSEK_	1.2602	0.40725
YIL038C	NOT3	_TPTTAAATTTSS(ph)NANSR_	1.2154	0.43042
YIL038C	NOT3	_TPTTAAATTTSSNANS(ph)R_	1.1417	0.47101
YIL041W	GVP36	_EFLSNSFAEEPEAKPEVAEEEKPQTAIS(ph)MNDEDDA_	1.6786	0.24041
YIL041W	GVP36	_EFLSNSFAEEPEAKPEVAEEEKPQT(ph)AISM(ox)NDEDDA_	1.611	0.2619
YIL041W	GVP36	_LTELTHATSAS(ph)EAQNILVAPGPIKEPK_	1.3991	0.34229
YIL106W	MOB1	_WNTANAANNAGSVS(ph)PTK_	0.86597	0.31861
YIL107C	PFK26	_S(ph)NPTSASSSQSELSEQPK_	1.5626	0.27842
YIL115C	NUP159	_S(ph)LSPTSEKIPIAGQEQEEK_	1.5664	0.27709
YIL115C	NUP159	_SLS(ph)PTSEKIPIAGQEQEEK_	1.4223	0.33246
YIL115C	NUP159	_DHNDDPKDGYVS(ph)GSEISVR_	1.106	0.4918
YIL122W	POG1	_DTNNDNNNHLTIPS(ph)PITTK_	2.4272	0.095105
YIL135C	VHS2	_TVSNNAANSLS(ph)R_	0.90421	0.35056
YIL135C	VHS2	_NFHNLS(ph)QR_	0.89469	0.34263
YIL135C	VHS2	_RPS(ph)TIGLDR_	0.86555	0.31826
YIL135C	VHS2	_SLSSQS(ph)FDNETSPAHPR_	0.7372	0.21225
YIL135C	VHS2	_SPS(ph)NQQYLLK_	0.53247	0.072208
YIL136W	OM45	_NNT(ph)GDANTEEAAAR_	2.9601	0.050962

YIL138C	TPM2	_YEEAQKELDEIANS(ph)LENL_	2.244	0.11875
YIL149C	MLP2	_KVKES(ph)PANDQASNE_	1.1094	0.4898
YIL154C	IMP2'	_DSNVSS(ph)LSR_	1.1713	0.45434
YIL154C	IMP2'	_DSNVS(ph)SLSR_	1.0434	0.463
YIR003W	AIM21	_KLS(ph)SPDTESK_	2.0726	0.14666
YIR003W	AIM21	_ANS(ph)EPPTPAGTPNVPTRRPILK_	2.0301	0.15459
YIR003W	AIM21	_ANS(ph)EPPT(ph)PAGTPNVPTR_	1.7841	0.21041
YIR003W	AIM21	_SNNEVT(ph)EHSDSEDLTEKQK_	1.1904	0.44385
YIR003W	AIM21	_IFQNPT(ph)DEESTTS(ph)LNEK_	1.1151	0.48645
YIR003W	AIM21	_SNNEVTEHS(ph)DSEDLTEK_	1.0897	0.49808
YIR003W	AIM21	_IFQNPT(ph)DEES(ph)TTSLNEK_	1.0664	0.48057
YIR003W	AIM21	_RST(ph)TEELNNVMNNTSK_	0.8027	0.26575
YIR003W	AIM21	_S(ph)TTEELNNVM(ox)NNTSK_	0.6661	0.15753
YIR003W	AIM21	_IFQNPT(ph)DEESTTSLNEK_	0.51346	0.062591
YIR006C	PAN1	_SVTESSPFVPSST(ph)PT(ph)PVDDR_	2.6776	0.070637
YIR006C	PAN1	_SVTESSPFVPSST(ph)PT(ph)PVDDR_	1.7144	0.22977
YIR006C	PAN1	_S(ph)SSPSYSQFK_	1.4266	0.33066
YIR006C	PAN1	_SVTESS(ph)PFVPSSTPTPVDDR_	1.1995	0.43896
YIR006C	PAN1	_GLDEDEDDGWS(ph)DEDESNNR_	1.1518	0.46527
YIR006C	PAN1	_NEEQSSFSS(ph)PSAK_	0.72185	0.20007
YJL005W	CYR1	_RSS(ph)IDADELDPM(ox)SPGPPSKK_	1.1368	0.47384
YJL020C	BBC1	_DLPEPISPET(ph)KK_	1.1592	0.46112
YJL020C	BBC1	_DLPEPIS(ph)PETKK_	1.1233	0.48163
YJL020C	BBC1	_STT(ph)HDVGEISNNVK_	1.0271	0.45033
YJL026W	RNR2	_AAADALS(ph)DLEIK_	0.75684	0.22807
YJL042W	MHP1	_IVT(ph)NNEEEVTVSK_	2.7383	0.0658
YJL042W	MHP1	_SPS(ph)PTHVDR_	0.99833	0.42755
YJL050W	MTR4	_NADTNVGDT(ph)PDHTQDKK_	1.5545	0.2813
YJL057C	IKS1	_RAS(ph)AGVESESSR_	0.90429	0.35063

YJL070C	YJL070C	_VLSENDGDVS(ph)PSVLK_	1.5723	0.27502
YJL076W	NET1	_KSS(ph)LETIVEK_	1.2872	0.3938
YJL076W	NET1	_SQS(ph)SIADNNGSPVK_	1.2787	0.398
YJL076W	NET1	_IVSNNS(ph)DDEDEDIGER_	0.91137	0.35653
YJL076W	NET1	_IVS(ph)NNSDDEDEDIGER_	0.80505	0.26771
YJL080C	SCP160	_LTYEPIDLSSILS(ph)DGEEKEVTKDTSNDSAK_	0.22173	0.00059918
YJL081C	ARP4	_KVT(ph)PTEEKEQEAVSK_	2.0173	0.15708
YJL082W	IML2	_S(ph)RTNDSSLLPGYMDSATLLHPGK_	2.0582	0.14929
YJL082W	IML2	_TNDS(ph)SLLPGYMDSATLLHPGK_	1.6425	0.25163
YJL082W	IML2	_LRDDLGLQTTPS(ph)QASDR_	1.6423	0.25171
YJL082W	IML2	_SRT(ph)NDSS(ph)LLPGYMDSATLLHPGK_	1.3503	0.36395
YJL082W	IML2	_SRT(ph)NDSSLLPGYM(ox)DSATLLHPGK_	1.1069	0.49126
YJL095W	BCK1	_NSKS(ph)QEDISNSR_	1.068	0.4818
YJL115W	ASF1	_KIEGGSTDIEST(ph)PK_	1.5877	0.26973
YJL115W	ASF1	_KIEGGS(ph)TDIESTPK_	1.5389	0.28692
YJL123C	MTC1	_KAPS(ph)ESVNNK_	3.2094	0.038511
YJL123C	MTC1	_KQKES(ph)EDEDEDDEIIDPSEWVK_	1.2383	0.41841
YJL123C	MTC1	_EADATPDDDRSSIS(ph)SNSNK_	1.0844	0.49418
YJL123C	MTC1	_EADATPDDDRSSISS(ph)NSNK_	0.9659	0.4014
YJL128C	PBS2	_TAQQPQQFAPSPS(ph)NKK_	1.0399	0.4603
YJL128C	PBS2	_SAS(ph)VGSNQSEQDKGSSQSPK_	0.90852	0.35415
YJL134W	LCB3	_TLS(ph)NPNDFQEPNYLLDPGNHPSDHFR_	0.86971	0.32175
YJL134W	LCB3	_T(ph)LSNPNDFQEPNYLLDPGNHPSDHFR_	0.78671	0.25252
YJL141C	YAK1	_NDLQPVLNAT(ph)PK_	1.1917	0.44314
YJL155C	FBP26	_LHQLLNDS(ph)PLEDKF_	2.8954	0.054873
YJL155C	FBP26	_GTSQVGELSQS(ph)STK_	2.6476	0.073175
YJL165C	HAL5	_NVDS(ph)GDEKDADASVNSGDDGDNDSEANM(ox)HK_	0.29701	0.0040793
YJL213W	YJL213W	_IMGGGGVAS(ph)PTDKISNK_	0.39815	0.020177
YJR001W	AVT1	_PEQEPLS(ph)PNGR_	0.71457	0.19435

YJR002W	MPP10	_HSS(ph)PDPYGINDK_	0.57158	0.094162
YJR007W	SUI2	_ELDNRSDS(ph)EDDEDES(ph)DDE_	0.98973	0.42066
YJR007W	SUI2	_ELDNRS(ph)DS(ph)EDDEDES(ph)DDE_	0.70753	0.18887
YJR049C	UTR1	_KISS(ph)ESSSR_	1.027	0.45025
YJR059W	PTK2	_SLGSPSVSSS(ph)K_	288.41	2.9032E-20
YJR059W	PTK2	_KRPT(ph)SPSISGSGSGGNSPSSSAGAR_	1.8502	0.1936
YJR059W	PTK2	_KRPTSPSISGSGS(ph)GGNSPSSSAGAR_	1.7121	0.23043
YJR059W	PTK2	_NFSAPNVSSSSNS(ph)LR_	1.3911	0.34575
YJR059W	PTK2	_NFSAPNVSSS(ph)SNSLR_	1.3287	0.37392
YJR059W	PTK2	_S(ph)LGSPSVSSSK_	1.1664	0.45711
YJR059W	PTK2	_S(ph)M(ox)LNSTPTTPT(ph)HNGPTPLPAK_	1.1481	0.46737
YJR059W	PTK2	_SM(ox)LNST(ph)PT(ph)TPTHNGPTPLPAK_	1.1281	0.47889
YJR059W	PTK2	_SM(ox)LNS(ph)TPT(ph)TPTHNGPTPLPAK_	1.1253	0.48051
YJR059W	PTK2	_SM(ox)LNST(ph)PTT(ph)PTHNGPTPLPAK_	1.1237	0.48141
YJR059W	PTK2	_SM(ox)LNST(ph)PTT(ph)PTHNGPTPLPAK_	1.1194	0.48394
YJR059W	PTK2	_VEDNLS(ph)EDDSTM(ox)K_	1.0748	0.48696
YJR072C	NPA3	_TASS(ph)ETAENIAK_	2.843	0.05828
YJR072C	NPA3	_GEVNENS(ph)APDLQR_	2.4386	0.093805
YJR072C	NPA3	_SSAAASDNDSIDAIS(ph)DLEEDANDGLVDRDEDEGVER_	0.96643	0.40183
YJR076C	CDC11	_RNT(ph)NPFK_	0.92578	0.36848
YJR091C	JSN1	_GSSDS(ph)FNLPHQISR_	1.09	0.49837
YJR104C	SOD1	_FEQASESEPTTVSYEIAGNS(ph)PNAER_	1.8007	0.20606
YKL021C	MAK11	_DAETADIGDQS(ph)EVESDTEELKK_	1.0415	0.4615
YKL021C	MAK11	_DAETADIGDQSEVESDT(ph)EELKK_	0.67993	0.1678
YKL021C	MAK11	_DAETADIGDQSEVES(ph)DTEELKK_	0.64849	0.14478
YKL022C	CDC16	_NS(ph)M(ox)FGSTIPST(ph)LRKVSLQR_	0.15436	0.000035188
YKL039W	PTM1	_GIDEDDLNLNFT(ph)DDEEGHDNVNNHSQGHGPVSPSPTK_	0.96107	0.39747
YKL042W	SPC42	_VKPENNMSETFAT(ph)PTPNNR_	1.0805	0.49128
YKL042W	SPC42	_VKPENNM(ox)SETFATPT(ph)PNNR_	0.97353	0.40759

YKL054C	DEF1	_KTES(ph)PLENVAELKK_	2.0217	0.15623
YKL054C	DEF1	_KT(ph)ESPLENVAELKK_	1.3178	0.37905
YKL054C	DEF1	_EQVKEEEQT(ph)AEELEQEQDNVAAPEEEVTVVEEK_	1.2992	0.38796
YKL054C	DEF1	_NNYNYYQTQNGQEQQS(ph)PNQGVAQHSEDSQQK_	1.2852	0.39476
YKL060C	FBA1	_DYIM(ox)S(ph)PVGNPEGPEKPNKK_	1.4543	0.31929
YKL064W	MNR2	_SFVDENSPT(ph)DRR_	2.2113	0.12361
YKL112W	ABF1	_ADDEEDLS(ph)DENIQPELR_	0.72205	0.20023
YKL112W	ABF1	_SNS(ph)IDYAK_	0.62043	0.12529
YKL129C	MYO3	_RGS(ph)VYHVPLNPVQATAVR_	0.86406	0.31702
YKL140W	TGL1	_QLDANSS(ph)TTALDALNKE_	1.0076	0.43492
YKL146W	AVT3	_NNNGGSTGISHASGS(ph)PLTDGNGGNSNGNSR_	1.9292	0.17534
YKL160W	ELF1	_GRGALVDS(ph)DDE_	0.99368	0.42383
YKL160W	ELF1	_TQNDGEIDS(ph)DEEEVDS(ph)DEER_	0.9398	0.38004
YKL175W	ZRT3	_RKS(ph)EGECCDLNK_	0.98741	0.4188
YKL186C	MTR2	_M(ox)GQDATVPIQPNNT(ph)GNR_	1.2764	0.39912
YKL204W	EAP1	_SATSLPSLDNNNQVPSSNVSVVNNDGNS(ph)TPHQSGSR_	0.76398	0.23388
YKL204W	EAP1	_SATSLPSLDNNNQVPSSNVS(ph)VVNNDGNSTPHQSGSR_	0.7531	0.22504
YKR001C	VPS1	_LAALES(ph)PPPVLK_	1.1847	0.44695
YKR007W	MEH1	_TNTFTLLTS(ph)PDSAK_	0.68886	0.17453
YKR018C	YKR018C	_LSGAHIGNS(ph)PAINR_	3.4745	0.028817
YKR018C	YKR018C	_DSSNSEDS(ph)EDEEMDGPTLLHPGK_	1.3376	0.36978
YKR028W	SAP190	_NYYNNVETNDDDYDS(ph)DDGKSK_	0.84781	0.30341
YKR048C	NAP1	_SSMQIDNAPT(ph)PHNT(ph)PASVLNPSYLK_	1.3576	0.36062
YKR048C	NAP1	_AQNDS(ph)EEEQVK_	1.3223	0.37691
YKR048C	NAP1	_SSMQIDNAPT(ph)PHNT(ph)PASVLNPSYLK_	0.88889	0.33779
YKR056W	TRM2	_LSS(ph)PLTDSGNR_	0.85026	0.30546
YKR067W	GPT2	_DGYDVSS(ph)DAESSISR_	1.0237	0.44761
YKR067W	GPT2	_RDGYDVS(ph)SDAESSISR_	0.65118	0.1467
YKR069W	MET1	_LSDIKLEDFETSS(ph)SPNKK_	1.0179	0.44309

YKR071C	DRE2	_VVDDLIEDS(ph)DDDDFSSDSSK_	0.35632	0.011435
YKR092C	SRP40	_ARES(ph)DNEDAKETK_	0.93918	0.37954
YKR093W	PTR2	_DSYVSDDVANS(ph)TER_	0.087262	1.4206E-07
YLL008W	DRS1	_GGKDDEIDEEDDS(ph)EEAK_	1.4054	0.33959
YLL013C	PUF3	_SVS(ph)NASLDTQNTFEQNVESDKNFNKLNR_	0.9153	0.35979
YLL018C	DPS1	_AVEES(ph)AEPAQVILGEDGKPLSKK_	6.0594	0.0024704
YLL021W	SPA2	_KLASSGEVDKIES(ph)PR_	1.0775	0.489
YLL021W	SPA2	_KPASFLNDVEEEES(ph)PVKPLK_	1.0118	0.43826
YLL021W	SPA2	_NFQEPLGNVES(ph)PDMTQK_	0.86063	0.31415
YLL024C	SSA2	_NTIS(ph)EAGDKLEQADKDAVTK_	1.2982	0.38844
YLL028W	TPO1	_TTT(ph)M(ox)NSAAES(ph)EVNITR_	0.936	0.37691
YLL032C	YLL032C	_TTIDNTSQSGAS(ph)PQR_	1.158	0.46177
YLL040C	VPS13	_KLAVLDPS(ph)ILGER_	1.8732	0.18809
YLL040C	VPS13	_SPS(ph)PDPASLSSESER_	1.6619	0.24553
YLL043W	FPS1	_TTGAQTNM(ox)ESNES(ph)PR_	2.4469	0.092881
YLL043W	FPS1	_NADDAHT(ph)IPESHLSR_	0.60799	0.117
YLL043W	FPS1	_ATSNAGHS(ph)ANTGATNGR_	0.51001	0.060922
YLL048C	YBT1	_ETSNEASSTNS(ph)ENVNK_	0.75966	0.23036
YLL048C	YBT1	_ETSNEAS(ph)S(ph)TNSENVNK_	0.66154	0.15419
YLL048C	YBT1	_ANS(ph)SANLAAK_	0.65265	0.14776
YLL055W	YCT1	_EAMKDY(ph)IT(ph)WLFGLFFLLQQLANNLPYQQNLLFEGM(ox)GGVDALGS(ph)T(ph)LVSVAGAGFAVVCAFIAT(ph)LMLAKWK_	21.348	0.000000539
YLR006C	SSK1	_DGNSS(ph)PQEFK_	1.3291	0.37373
YLR006C	SSK1	_GKDGNS(ph)SPQEFK_	0.95914	0.3959
YLR018C	POM34	_HSGISSTLVSANNDNNSPHT(ph)PVTR_	2.2903	0.11223
YLR025W	SNF7	_IKQSENS(ph)VKDGEEEEDEEDEDEK_	1.2282	0.42369
YLR044C	PDC1	_NPVILADACCS(ph)R_	0.94299	0.38267
YLR045C	STU2	_VASS(ph)PLRNDNK_	1.0637	0.47855

YLR045C	STU2	_VAS(ph)SPLRNDNK_	1.0266	0.44993
YLR058C	SHM2	_LITSHLVDTDPEVDS(ph)IIKDEIER_	0.60027	0.11198
YLR075W	RPL10	_KGS(ph)LENNIR_	0.94344	0.38303
YLR079W	SIC1	_SQES(ph)EDEEDIIINPVR_	0.76244	0.23262
YLR096W	KIN2	_NES(ph)EILER_	1.1701	0.45504
YLR150W	STM1	_ELS(ph)AEKEAQADAAAEIAEDAAEAEDAGKPK_	3.1863	0.039507
YLR175W	CBF5	_VNENT(ph)PEQWKK_	2.3946	0.098905
YLR175W	CBF5	_EYVPLDNAEQSTSSS(ph)QETK_	0.84368	0.29994
YLR177W	YLR177W	_RNT(ph)QPVLNLHPAAAPTNDAGLAVVDGK_	0.89893	0.34616
YLR177W	YLR177W	_RNTQPVLNLHPAAAPT(ph)NDAGLAVVDGK_	0.85624	0.31047
YLR181C	VTA1	_EIGGES(ph)EAEDSDK_	0.6157	0.12211
YLR187W	SKG3	_VFKNDESPSTPS(ph)SPK_	1.5241	0.29231
YLR187W	SKG3	_VFKNDESPS(ph)TPSSPK_	1.2085	0.43412
YLR196W	PWP1	_ATLEEAEGES(ph)GVEDDAATGSSNK_	0.86801	0.32033
YLR206W	ENT2	_SNPHDSS(ph)PSYQDDLEK_	1.9355	0.17396
YLR206W	ENT2	_TGTFINSQGT(ph)GYK_	0.8413	0.29795
YLR219W	MSC3	_NVYHTDAAS(ph)DNASAPLGSNK_	2.1223	0.13792
YLR219W	MSC3	_NVYHT(ph)DAASDNASAPLGSNK_	1.7856	0.21002
YLR219W	MSC3	_STAGNNNDS(ph)RANSIT(ph)VK_	1.5802	0.2723
YLR219W	MSC3	_STAGNNNDS(ph)RANSITVK_	0.97447	0.40836
YLR220W	CCC1	_NSAQDLENSPMS(ph)VGKDNR_	1.165	0.45785
YLR231C	BNA5	_KRSLLLTNYM(ox)T(ph)ELLEAS(ph)K_	0.13786	0.000013073
YLR248W	RCK2	_NSS(ph)NEFLTK_	0.7859	0.25185
YLR248W	RCK2	_DVSQITSS(ph)PK_	0.48761	0.050675
YLR249W	YEF3	_KKELGDAYVSS(ph)DEEF_	1.2023	0.43744
YLR249W	YEF3	_KKELGDAYVS(ph)SDEEF_	0.95217	0.3902
YLR257W	YLR257W	_AS(ph)KSNSLITSTDPVEDHISK_	3.2656	0.036188
YLR257W	YLR257W	_GS(ph)TPCLIGDSIR_	2.684	0.070107
YLR257W	YLR257W	_GES(ph)YQSAEQEIDHTAPEKSEK_	2.3622	0.10285

YLR257W	YLR257W	_DRS(ph)YTSVAELNR_	2.0378	0.15313
YLR257W	YLR257W	_DRS(ph)YTSVAELNR_	1.7732	0.21332
YLR257W	YLR257W	_NKS(ph)INSESFSSPSLR_	1.6852	0.23841
YLR257W	YLR257W	_TVS(ph)PSKGEDSR_	1.2221	0.42686
YLR258W	GSY2	_VARPLSVPGS(ph)PR_	2.9792	0.049865
YLR258W	GSY2	_VARPLS(ph)VPGSPR_	2.7412	0.06558
YLR259C	HSP60	_RGS(ph)QVAVEK_	0.43323	0.030154
YLR309C	IMH1	_S(ph)GSIGTLANANIDSSPANNSNPTKLEK_	0.66462	0.15645
YLR318W	EST2	_LFNVVNAS(ph)R_	1.1133	0.48749
YLR319C	BUD6	_RLS(ph)SVVTTSPDK_	0.44868	0.035352
YLR330W	CHS5	_DATESVAVEPSNEDVKPEEKGS(ph)EAEDDINNVSK_	1.9319	0.17475
YLR330W	CHS5	_DATESVAVEPS(ph)NEDVKPEEK_	1.1652	0.45776
YLR335W	NUP2	_ADGTGEAQVDNS(ph)PTTESNSR_	2.2931	0.11185
YLR335W	NUP2	_ADGTGEAQVDNSPT(ph)TESNSR_	1.9584	0.16905
YLR335W	NUP2	_ETYDSNES(ph)DDDVTPSTK_	1.0209	0.44545
YLR335W	NUP2	_ETYDS(ph)NES(ph)DDDVTPSTK_	0.60448	0.11471
YLR345W	YLR345W	_PNVLS(ph)DDEELLNGLGSEIMKPSR_	0.64353	0.14125
YLR356W	ATG33	_LAASELSDS(ph)IIDLGEDNHASENTPR_	4.7212	0.0081642
YLR356W	ATG33	_LAASELS(ph)DSIIDLGEDNHASENTPR_	4.3362	0.011851
YLR371W	ROM2	_YTS(ph)VSGT(ph)SLSSPR_	1.0027	0.43106
YLR382C	NAM2	_QDTLNSGS(ph)K_	0.83433	0.29212
YLR399C	BDF1	_RSS(ph)AQEDAPIVIR_	1.0586	0.47469
YLR399C	BDF1	_DASS(ph)LS(ph)PTSAGSR_	0.75326	0.22517
YLR399C	BDF1	_WADRPNLDDYDS(ph)DEDSR_	0.71802	0.19706
YLR399C	BDF1	_DASS(ph)LS(ph)PTSAGSR_	0.66802	0.15894
YLR399C	BDF1	_RS(ph)SAQEDAPIVIR_	0.59595	0.10921
YLR405W	DUS4	_SAS(ph)VVERQE_	0.39561	0.019551
YLR410W-B	YLR410W-B	_NVSRTS(ph)PNTTNTK_	0.72502	0.20257
YLR410W-B	YLR410W-B	_ESTEM(ox)GGTIESDTTS(ph)PR_	0.27765	0.0026984

YLR413W	YLR413W	_IIEEHES(ph)PIDAEK_	0.076251	3.1918E-08
YLR413W	YLR413W	_AVQESDS(ph)TTSR_	0.062093	2.856E-09
YLR421C	RPN13	_M(ox)IGVLNNSSES(ph)DEEESNDEK_	0.62371	0.12751
YLR429W	CRN1	_SSDIDQVNNAEDPSRDTSGWEEADDEPAPIKIETPVT(ph)PTETK_	1.6993	0.23419
YLR429W	CRN1	_SASS(ph)SSTINHVLKEDNSINK_	1.3041	0.38557
YLR429W	CRN1	_DTSGWEEADDEPAPIKIET(ph)PVTPTETKK_	1.1116	0.48849
YLR429W	CRN1	_SASSSS(ph)TINHVLKEDNSINK_	1.0003	0.42913
YML008C	ERG6	_KPENAETPS(ph)QTSQEATQ_	1.8859	0.18512
YML008C	ERG6	_KPENAETPSQTS(ph)QEATQ_	1.7813	0.21116
YML008C	ERG6	_KPENAET(ph)PSQTSQEATQ_	1.1569	0.46242
YML029W	USA1	_S(ph)QSPVSFAPTQGR_	0.9809	0.41355
YML034W	SRC1	_HLNLLSSDS(ph)EIEQDYQK_	2.2988	0.11108
YML034W	SRC1	_HLNLLS(ph)SDSEIEQDYQK_	1.9169	0.17806
YML035C	AMD1	_KIGDEQAGVVVDDET(ph)PPLEQQDSHESLAADSR_	3.5797	0.025744
YML035C	AMD1	_KIGDEQAGVVVDDETPPLEQQDS(ph)HESLAADSR_	1.7754	0.21273
YML035C	AMD1	_LNDLSLEPAPSHDEQDGS(ph)GLVIDIDQR_	0.9877	0.41903
YML052W	SUR7	_SHERPDDVS(ph)V_	0.92637	0.36896
YML052W	SUR7	_EKEQATDPILTATGPEDMQQS(ph)ASIVGPSSNANPVTATAATENQPK_	0.79557	0.25985
YML057W	CMP2	_TERPQSSTT(ph)PIDSK_	2.9359	0.052388
YML057W	CMP2	_SEATPQPATSAS(ph)PK_	1.4213	0.33287
YML062C	MFT1	_DGLLNEAEGDNIDEDYES(ph)DEDEERKER_	1.0103	0.43709
YML062C	MFT1	_DGLLNEAEGDNIDEDY(ph)ESDEDEERKER_	0.60797	0.11699
YML072C	TCB3	_TSNS(ph)VSDVSK_	1.5363	0.28785
YML072C	TCB3	_SPSNLNSTSVT(ph)PR_	1.023	0.44708
YML093W	UTP14	_TAQSNGNDDEDAS(ph)PQLK_	0.87053	0.32243
YML100W	TSL1	_IAS(ph)PIQHEHDSGSR_	9.1531	0.00024373
YML101C	CUE4	_TVQDAKPAPSVATNDPS(ph)PEPVPSAPEER_	3.2151	0.038265

YML111W	BUL2	_SAS(ph)TTNLDR_	0.96843	0.40346
YML111W	BUL2	_IDDTASQS(ph)PSYDSK_	0.92652	0.36909
YML111W	BUL2	_ASDSQDDDIRSASTT(ph)NLDR_	0.89209	0.34046
YML112W	CTK3	_DSITSSSTTT(ph)PPSSQQK_	0.69911	0.18236
YML112W	CTK3	_DSITSS(ph)ST(ph)TTPPSSQQK_	0.65187	0.1472
YML115C	VAN1	_AM(ox)DNGLSLPIS(ph)R_	1.1162	0.48581
YML117W	NAB6	_SSS(ph)QTVINSK_	1.8685	0.18921
YMR004W	MVP1	_HLIALPS(ph)TSPSEER_	1.6162	0.26018
YMR005W	TAF4	_ANS(ph)PKKPSDGTGVSASDTPK_	1.0135	0.43963
YMR010W	YMR010W	_ALKQDSNDT(ph)SDSPQDDQVGK_	1.8104	0.20356
YMR012W	CLU1	_DDVKPELANKS(ph)VDELLTFIEGDSSNSK_	2.4121	0.096841
YMR014W	BUD22	_ETTS(ph)DNEDLLIK_	0.58634	0.10316
YMR031C	EIS1	_NNS(ph)ITSATSK_	0.79639	0.26052
YMR031C	EIS1	_EATETSSAVQTKEPEEKIS(ph)IGNK_	0.73529	0.21073
YMR031C	EIS1	_EATETSS(ph)AVQTK_	0.72291	0.20091
YMR031C	EIS1	_NNSITS(ph)ATSK_	0.54239	0.077507
YMR039C	SUB1	_LLS(ph)DDEYEDDNNNDSTNNDKDKNGK_	0.98693	0.41841
YMR039C	SUB1	_LLSDDEY(ph)EDDNNNDSTNNDKDKNGK_	0.81613	0.27693
YMR049C	ERB1	_RAASEES(ph)DVEEDEDK_	0.7835	0.24988
YMR054W	STV1	_NQS(ph)VEDLSFLEQGYQHR_	1.3436	0.36701
YMR086W	SEG1	_NLENDTTSS(ph)PTQDLDEK_	1.2555	0.40963
YMR104C	YPK2	_SKEDDGSS(ph)EDENEK_	4.2998	0.012285
YMR104C	YPK2	_KASQSST(ph)ETQGPSSESGLM(ox)TVK_	2.3243	0.10769
YMR109W	MYO5	_RGS(ph)VYHVPLNIVQADAVR_	0.84702	0.30274
YMR111C	YMR111C	_IKPEPGLSDFENGEYDGNES(ph)DENATTR_	1.0708	0.48393
YMR139W	RIM11	_QLKPTEPNVSY(ph)ICSR_	0.93111	0.37288
YMR140W	SIP5	_NT(ph)SHSITPIHDESTSASR_	0.79682	0.26088
YMR152W	YIM1	_DFVSLS(ph)S(ph)ILKAINPFK_	1.3907	0.34594
YMR192W	GYL1	_IALRDEIS(ph)VPEGDEK_	1.5384	0.28709

YMR196W	YMR196W	_S(ph)SVATTASTESSEQGPK_	1.2692	0.4027
YMR196W	YMR196W	_IGGTHSGLT(ph)PQSSISSDK_	1.1326	0.47629
YMR196W	YMR196W	_IGGTHSGLTPQSSIS(ph)SDKAR_	0.5598	0.087254
YMR196W	YMR196W	_SSVATTAS(ph)TES(ph)SEQGPK_	0.23781	0.00097728
YMR205C	PFK2	_VHS(ph)YTDLAYR_	0.83526	0.2929
YMR205C	PFK2	_VHSYT(ph)DLAYR_	0.79357	0.25818
YMR212C	EFR3	_DNQIST(ph)SDLLSDSQVR_	1.2641	0.40528
YMR212C	EFR3	_TATGENQNDDFKDANEDLHS(ph)LSSR_	0.97246	0.40673
YMR216C	SKY1	_NSNNS(ph)FLNSVPHSVTR_	1.1374	0.47353
YMR219W	ESC1	_SVESDLHEHS(ph)PDNLYDLAAR_	1.0003	0.42911
YMR219W	ESC1	_NLS(ph)DLENYSQR_	0.56631	0.091044
YMR221C	YMR221C	_LIEGDTGS(ph)GIIPDEQER_	1.0326	0.45459
YMR224C	MRE11	_TGS(ph)PDITQSHVDNESR_	2.0329	0.15406
YMR261C	TPS3	_VCS(ph)PSQEASASSISASR_	1.7079	0.23167
YMR261C	TPS3	_NPNLSFDS(ph)HPPR_	1.6509	0.24899
YMR285C	NGL2	_DQPES(ph)PVPEKFHANEEQSELVDK_	1.9568	0.16939
YMR290C	HAS1	_SRDS(ph)ESTEEPVVDEK_	1.0785	0.48977
YMR295C	YMR295C	_KKS(ph)SISNTSDHDGANR_	1.1553	0.46332
YMR295C	YMR295C	_KSS(ph)ISNTSDHDGANR_	0.74976	0.22235
YMR295C	YMR295C	_SSISNTS(ph)DHDGANR_	0.56028	0.087532
YMR300C	ADE4	_RES(ph)IANNSSDM(ox)K_	0.49024	0.051826
YMR311C	GLC8	_ENKQPDFETNDENDEDS(ph)PEAR_	0.87358	0.32498
YMR311C	GLC8	_GGILKNPLALS(ph)PEQLAQQDPETLEEFRR_	0.10413	8.9962E-07
YNL004W	HRB1	_ESVHNHS(ph)DGDDVDIPM(ox)DDSPVNEEAR_	0.91923	0.36305
YNL015W	PBI2	_HNDVIENVEEDKEVHT(ph)N_	4.3135	0.01212
YNL027W	CRZ1	_SIS(ph)PDEKAK_	0.81309	0.2744
YNL035C	YNL035C	_KMNVLGDDDREGS(ph)INLDEPLIIQK_	3.3937	0.031451
YNL039W	BDP1	_EIEEDNS(ph)DNDKGVDENETAIVEKPSLVGER_	0.59107	0.10612
YNL069C	RPL16B	_KVSSASAAAS(ph)ESDVAK_	1.2636	0.4055

YNL074C	MLF3	_SNSNNSNPS(ph)FIFER_	1.0111	0.43772
YNL074C	MLF3	_SNSNNS(ph)NPSFIFER_	0.82697	0.28597
YNL074C	MLF3	_T(ph)NSATLPSSESSPASPDLK_	0.7319	0.20803
YNL074C	MLF3	_KNSNNVS(ph)SPLDNVIPTSR_	0.70262	0.18507
YNL085W	MKT1	_S(ph)NLSSPSS(ph)ASSSASPATTVTK_	0.95284	0.39075
YNL085W	MKT1	_SNLSSPSSAS(ph)SSAS(ph)PATTVTK_	0.70285	0.18525
YNL088W	TOP2	_M(ox)GSTSATS(ph)KENT(ph)PEQDDVATKK_	1.9986	0.16079
YNL088W	TOP2	_MGSTS(ph)ATSKENT(ph)PEQDDVATKK_	1.9826	0.16402
YNL088W	TOP2	_MGSTS(ph)ATSKENT(ph)PEQDDVATKK_	1.9667	0.1673
YNL098C	RAS2	_QAINVEEAFY(ph)T(ph)LARLVR_	11.479	0.000056917
YNL098C	RAS2	_NVNSS(ph)TTVVNAR_	2.7132	0.067755
YNL098C	RAS2	_GSGANSVPRNS(ph)GGHR_	1.5662	0.27717
YNL112W	DBP2	_DRSDS(ph)EIAQFR_	0.74216	0.21623
YNL113W	RPC19	_HIQEEEEQDVDMT(ph)GDEEQEEEPDREK_	0.78962	0.25492
YNL118C	DCP2	_NPISSTVSSNQQS(ph)PK_	0.35102	0.010554
YNL118C	DCP2	_RNS(ph)VSKPQNSEENASTSSINDANASELLGMLK_	0.20156	0.00029844
YNL121C	TOM70	_RAS(ph)ANEGLGK_	3.0275	0.047206
YNL121C	TOM70	_FGDIDTATATPT(ph)ELSTQPAK_	2.1466	0.13384
YNL121C	TOM70	_FGDIDTATAT(ph)PTELSTQPAKER_	1.6825	0.23924
YNL121C	TOM70	_STAPSNPPIYPVSS(ph)NGEPDFSNK_	1.4772	0.31021
YNL124W	NAF1	_NKGTDASNGYDEELPEEEQEFS(ph)DDEKEALFK_	0.81031	0.27208
YNL149C	PGA2	_RRNQGLDPDS(ph)DADIEELLEE_	0.68524	0.1718
YNL151C	RPC31	_LKELAEDVDDAS(ph)TGDGAAK_	0.82348	0.28306
YNL154C	YCK2	_YQLQPDDS(ph)HYDEEREASKLDPTSYEAYQQQTQQK_	1.2168	0.42969
YNL157W	IGO1	_KYFDS(ph)GDYALQK_	1.4986	0.3019
YNL166C	BNI5	_LADQTPHDDNSENCPNRS(ph)GGS(ph)TPLDSQTK_	1.1777	0.45079
YNL166C	BNI5	_SGGST(ph)PLDSQTK_	1.1187	0.48432
YNL166C	BNI5	_LADQT(ph)PHDDNSENCPNR_	0.98594	0.41761
YNL166C	BNI5	_LADQTPHDDNSENCPNRS(ph)GGS(ph)TPLDSQTK_	0.57697	0.097408

YNL173C	MDG1	_SIFSQEVVELPDS(ph)EDETQQVNK_	0.95899	0.39578
YNL175C	NOP13	_SGASEKDAQGEES(ph)TINT(ph)PTGDESGEVVK_	2.0049	0.15952
YNL178W	RPS3	_EEEPILAPS(ph)VK_	1.0442	0.46359
YNL183C	NPR1	_NKS(ph)SSHIGSVSNSSSSDR_	1.0275	0.45062
YNL183C	NPR1	_ASM(ox)DSNNANATQS(ph)R_	0.68041	0.16816
YNL197C	WHI3	_FM(ox)QQPQPEHM(ox)YPVNQSNT(ph)PQKVPPAR_	2.0971	0.14228
YNL206C	RTT106	_DNS(ph)FASINGQPEQELQYK_	1.0888	0.49745
YNL212W	VID27	_SSLTAS(ph)ADDLKEIEHR_	1.7493	0.21985
YNL231C	PDR16	_LLYPVKS(ph)ESST(ph)V_	2.1366	0.1355
YNL231C	PDR16	_LLYPVKSESS(ph)T(ph)V_	1.9173	0.17796
YNL231C	PDR16	_LLYPVKSESS(ph)TV_	0.82982	0.28836
YNL243W	SLA2	_T(ph)PTPTPPVVAEPAIS(ph)PRPVSQR_	0.29833	0.0041894
YNL246W	VPS75	_M(ox)M(ox)S(ph)DQENENEHAK_	0.75517	0.22672
YNL247W	YNL247W	_GQLDLINDGEGS(ph)LSNFADNGKK_	1.5029	0.30028
YNL271C	BNI1	_GGAENNTS(ph)AS(ph)TLPGDR_	1.0704	0.48365
YNL271C	BNI1	_GGAENNTS(ph)AST(ph)LPGDR_	1.0686	0.48226
YNL272C	SEC2	_IGPLVEDDS(ph)DEDQNDAISVR_	1.175	0.45229
YNL287W	SEC21	_SETTLDTT(ph)PEAESVPEKR_	3.3235	0.033958
YNL287W	SEC21	_ADANS(ph)FAGPNLDDHQEDLLATK_	2.0043	0.15965
YNL297C	MON2	_NISTSSVTT(ph)S(ph)PVESTKNPSR_	0.87674	0.32763
YNL307C	MCK1	_KLEHNQPSISY(ph)ICSR_	1.1728	0.4535
YNL308C	KRI1	_EVS(ph)PESFGLTAR_	0.64147	0.1398
YNL321W	VNX1	_SHSVPDLNTATPSS(ph)PK_	0.62961	0.13154
YNL323W	LEM3	_GAIVSGDNPEEEEDVDAS(ph)EFEEDEVKPVR_	0.91485	0.35941
YNL330C	RPD3	_DAEDLGDVEEDS(ph)AEAKDTK_	2.9606	0.050933
YNR002C	ATO2	_HSQES(ph)ICK_	0.17255	0.000088884
YNR016C	ACC1	_AVS(ph)VSDLSYVANSQSSPLR_	1.2464	0.41425
YNR019W	ARE2	_KSS(ph)PDAVDSVGK_	3.0341	0.046859
YNR019W	ARE2	_S(ph)SPDAVDSVGK_	1.2277	0.42392

YNR024W	MPP6	_DKEFTGS(ph)QDDGEDEYDLDKLFK_	0.99354	0.42372
YNR047W	FPK1	_T(ph)NSFVGTEEYIAPEVIR_	1.133	0.47606
YNR051C	BRE5	_EGS(ph)VEAINAVNNSSLPNGK_	1.8805	0.18639
YNR051C	BRE5	_ESGNNASTPS(ph)SSPEPVANPPK_	1.841	0.19586
YNR051C	BRE5	_ESGNNAST(ph)PSS(ph)SPEPVANPPK_	0.92034	0.36397
YNR051C	BRE5	_ESGNNAS(ph)TPSSS(ph)PEPVANPPK_	0.83041	0.28884
YNR052C	POP2	_QASEQHQQQNMGPQVYS(ph)PK_	1.0431	0.46274
YNR067C	DSE4	_VS(ph)AASHSPLSVSPK_	2.2911	0.11212
YOL004W	SIN3	_VTTPM(ox)GTTTVNNNIS(ph)PSGR_	1.223	0.42644
YOL004W	SIN3	_IECSSNPDDPIRVTT(ph)PM(ox)GTTTVNNNIS(ph)PSGR_	1.152	0.46517
YOL006C	TOP1	_IKTEPVQSSSLPS(ph)PPAKK_	0.76343	0.23343
YOL006C	TOP1	_VASMNSASLQDEAEPYDS(ph)DEAISK_	0.64898	0.14513
YOL016C	CMK2	_NMYSLGDDGDNDIEENS(ph)LNESLLDGVTHSLDDLR_	1.3172	0.37935
YOL041C	NOP12	_KLFGTNPIAETEES(ph)GNEKEEESSK_	0.80662	0.26901
YOL054W	PSH1	_HVIIT(ph)DDEEEEQRR_	1.5156	0.29549
YOL054W	PSH1	_NSALAVADDS(ph)DDGITR_	1.2261	0.42478
YOL054W	PSH1	_NYAGGRDEFDEEEYS(ph)EGELDEIR_	1.0414	0.46144
YOL059W	GPD2	_RS(ph)DSAVSIVHLK_	0.85023	0.30543
YOL059W	GPD2	_SDS(ph)AVSIVHLK_	0.75179	0.22398
YOL060C	MAM3	_AADQADESS(ph)PLLSPSNSNHPSEHPQQDLNNK_	2.2184	0.12253
YOL060C	MAM3	_TIIGPAKDWDESKS(ph)EYGNENINQENSNR_	1.0928	0.49965
YOL060C	MAM3	_AADQADESSPLLS(ph)PSNSNHPSEHPQQDLNNK_	0.55232	0.083
YOL086C	ADH1	_GLVKS(ph)PIKVVGLSTLPEIYEK_	3.6018	0.025145
YOL086C	ADH1	_SIS(ph)IVGSYVGNR_	0.571	0.093817
YOL109W	ZEO1	_NEAT(ph)PEAEQVKKEEQNIADGVEQK_	0.82189	0.28173
YOL109W	ZEO1	_EQAEAS(ph)IDNLK_	0.81658	0.2773
YOL109W	ZEO1	_LEETKES(ph)LQNK_	0.61278	0.12017
YOL137W	BSC6	_RNS(ph)QDEDSLPNNTNLIK_	0.91074	0.356
YOL139C	CDC33	_TVLSDS(ph)AHFDVKHPLNTK_	3.8632	0.019113

YOL139C	CDC33	_KFEENVSVDDTTAT(ph)PK_	1.2934	0.39076
YOL144W	NOP8	_LIEDS(ph)DNDIDHAK_	0.66587	0.15736
YOR027W	STI1	_KDAEPQS(ph)DSTTSKENSSK_	1.3423	0.36759
YOR039W	CKB2	_SENVGTVT(ph)R_	2.1731	0.12953
YOR042W	CUE5	_DGT(ph)PKVEEK_	3.4711	0.028924
YOR042W	CUE5	_RQT(ph)QLEQDELLAR_	2.7156	0.067565
YOR042W	CUE5	_SNVPES(ph)INEDISK_	1.9582	0.1691
YOR042W	CUE5	_VVAETTYIDT(ph)PDT(ph)ETK_	1.7779	0.21207
YOR042W	CUE5	_TTDVDLNS(ph)DGKKDNDTSAK_	1.6582	0.2467
YOR042W	CUE5	_EQHHEDS(ph)EEEDSWSQFVEK_	1.6245	0.25745
YOR042W	CUE5	_VVAETTYIDT(ph)PDTETK_	1.4369	0.32637
YOR042W	CUE5	_NKKNPDEDEFLINS(ph)DDEM_	1.3868	0.34765
YOR042W	CUE5	_WQPLPPEPLDTT(ph)PTKVNAVSR_	1.1807	0.44919
YOR046C	DBP5	_LADIQADPNS(ph)PLYSAK_	1.3535	0.36247
YOR051C	ETT1	_KSDEPS(ph)REST(ph)PVR_	1.0532	0.47053
YOR051C	ETT1	_KSDEPS(ph)REST(ph)PVR_	1.01	0.43679
YOR051C	ETT1	_S(ph)DEPSREST(ph)PVR_	0.66763	0.15866
YOR078W	BUD21	_HVTFDKLDES(ph)DENEEALAK_	0.92255	0.3658
YOR089C	VPS21	_TAEEQNS(ph)ASNER_	1.4474	0.3221
YOR089C	VPS21	_TAEEQNSASNERES(ph)NNQR_	0.96207	0.39829
YOR092W	ECM3	_TNHVDAQS(ph)VSELNDPTYR_	1.367	0.35638
YOR124C	UBP2	_KKNESNDAEVS(ph)ENEDTTGLTSPTR_	1.8985	0.18221
YOR140W	SFL1	_SLGSTSS(ph)LPNDR_	1.1233	0.48165
YOR140W	SFL1	_SSSTT(ph)NIPSR_	0.93995	0.38017
YOR140W	SFL1	_NSS(ph)GDENTGGGVQEK_	0.65545	0.14977
YOR140W	SFL1	_KNSS(ph)NQNYDIDSGAR_	0.49575	0.054278
YOR141C	ARP8	_APPAVQT(ph)SK_	1.7957	0.20737
YOR145C	PNO1	_IIGINNTESIDEDDDDDVLLDDS(ph)DNNTAKEEVEGEEGSR_	0.13812	0.000013296
YOR153W	PDR5	_NANDPENVGERS(ph)DLSSDRK_	0.42381	0.027226

YOR171C	LCB4	_GCLTFEPNPS(ph)PNSS(ph)PDLLSK_	0.54155	0.077053
YOR175C	ALE1	_DISASS(ph)PNLGGILK_	0.69335	0.17795
YOR189W	IES4	_IEDTSPPS(ph)ANSR_	2.6104	0.076451
YOR189W	IES4	_IEDTS(ph)PPSANSR_	1.4445	0.32325
YOR198C	BFR1	_KVVADDLVLVT(ph)PK_	1.517	0.29498
YOR204W	DED1	_DAMMS(ph)APGS(ph)R_	2.0617	0.14865
YOR204W	DED1	_DAMM(ox)SAPGS(ph)R_	1.4363	0.32661
YOR204W	DED1	_VGS(ph)TSENITQK_	1.4343	0.32745
YOR206W	NOC2	_YLEENDKDLLDFAGTNPLDGIDS(ph)QDEGEDAER_	0.61455	0.12135
YOR216C	RUD3	_M(ox)STDPEADGIVAS(ph)PDDEGKDLSEGVDKQK_	1.8017	0.2058
YOR217W	RFC1	_KMPVSNVIDVSET(ph)PEGEK_	2.3853	0.10003
YOR217W	RFC1	_QVGSSKPEVIDLDTES(ph)DQESTNKTPK_	0.93535	0.37638
YOR220W	RCN2	_SSQTS(ph)LPSQLENKDK_	2.4557	0.091906
YOR220W	RCN2	_NKPLLSINT(ph)DPGVTGVDSSSLNK_	2.3849	0.10008
YOR220W	RCN2	_GGSSLSPDKSSLES(ph)PTMLK_	1.623	0.25794
YOR220W	RCN2	_SSS(ph)STSNLSLNR_	1.2659	0.40436
YOR220W	RCN2	_SRS(ph)TDDAVSLQDNNLALLEDHR_	1.2243	0.42573
YOR220W	RCN2	_GGSSLS(ph)PDKSSLES(ph)PTM(ox)LK_	0.79209	0.25696
YOR227W	HER1	_IS(ph)TPTST(ph)PTTASSKPSSSGGNR_	1.0152	0.44096
YOR229W	WTM2	_S(ph)AATPNPEYGDAFQDVEGKPLRPK_	4.7505	0.0079408
YOR239W	ABP140	_TAEKPLETNLPKPETNEEDEEEGS(ph)M(ox)SENK_	1.218	0.42906
YOR239W	ABP140	_TAEKPLETNLPKPET(ph)NEEDEEEGSM(ox)SENK_	1.0918	0.49971
YOR239W	ABP140	_SDFEKS(ph)DTEGSR_	0.75045	0.2229
YOR261C	RPN8	_VSDDS(ph)ESESGDKEAT(ph)APLIQR_	2.5507	0.082055
YOR261C	RPN8	_VSDDSES(ph)ESGDKEATAPLIQR_	1.502	0.30063
YOR261C	RPN8	_VSDDS(ph)ESESGDKEATAPLIQR_	1.4073	0.33877
YOR265W	RBL2	_S(ph)AITSAQELLDSK_	1.456	0.3186
YOR267C	HRK1	_FLNHSDCS(ph)AINQQQPAHESNLK_	2.6473	0.073198
YOR267C	HRK1	_ST(ph)STVNLNNHYR_	0.63987	0.13868

YOR267C	HRK1	_STS(ph)TVNLNNHYR_	0.52344	0.067553
YOR273C	TPO4	_PSSLT(ph)KTES(ph)NSDPR_	0.2933	0.0037818
YOR276W	CAF20	_KKGS(ph)GEDDEEETETTPTSTVPVATIAQETLK_	1.4145	0.33571
YOR276W	CAF20	_KKGS(ph)GEDDEEETETTPTSTVPVATIAQETLK_	1.1077	0.4908
YOR276W	CAF20	_KKGSGEDDEEETET(ph)TPTSTVPVATIAQETLK_	0.85175	0.3067
YOR276W	CAF20	_GSGEDDEEETETT(ph)PTSTVPVATIAQETLK_	0.80877	0.2708
YOR276W	CAF20	_DIVADKPILGFNAFAALES(ph)EDEDDEA_	0.60842	0.11729
YOR287C	RRP36	_EIDEQES(ph)S(ph)DDELK_	0.37274	0.014479
YOR290C	SNF2	_NAPLDSKDENFASVS(ph)PAGPSSVHNAK_	0.9559	0.39325
YOR298C-A	MBF1	_LRGNNIGS(ph)PLGAPK_	1.5973	0.26646
YOR304W	ISW2	_TSATREDT(ph)PLSQNESTR_	1.7087	0.23142
YOR304W	ISW2	_NEGS(ph)DAEEEEGEYKNAANTEGHK_	1.0922	0.5
YOR308C	SNU66	_VKVNFDS(ph)ANNM(ox)SDEDGGDFKPLK_	1.0807	0.49141
YOR352W	TFB6	_RLS(ph)M(ox)SQQSK_	0.65737	0.15116
YPL004C	LSP1	_ALLELLDDS(ph)PVTPGEAR_	2.0541	0.15006
YPL009C	TAE2	_HCTISS(ph)DTDSDSGNAK_	0.7852	0.25127
YPL015C	HST2	_LNGHDS(ph)DEDGASNSSSSQK_	1.0367	0.45775
YPL019C	VTC3	_SNSLSSDGNS(ph)NQDVEIGK_	0.95014	0.38854
YPL030W	TRM44	_KFEIDNGNES(ph)GEEDVKK_	0.87353	0.32494
YPL032C	SVL3	_KPS(ph)FPQLQQSANVR_	0.98765	0.41899
YPL049C	DIG1	_VNDSYDS(ph)PLSGTASTGK_	0.75396	0.22574
YPL058C	PDR12	_S(ph)LLHYLR_	2.5303	0.084068
YPL058C	PDR12	_KEMDS(ph)FEINDLDFDLR_	2.4345	0.094272
YPL058C	PDR12	_SNHDDDYANSVQS(ph)YAASEGQVDNEDLAATSQLSR_	1.4588	0.31748
YPL058C	PDR12	_SNHDDDYANS(ph)VQSYAASEGQVDNEDLAATSQLSR_	1.3151	0.38033
YPL058C	PDR12	_SNHDDDYANSVQSYAAS(ph)EGQVDNEDLAATSQLSR_	1.301	0.38709
YPL058C	PDR12	_HLSNILS(ph)NEEGIER_	1.0521	0.46971
YPL058C	PDR12	_HLS(ph)NILSNEEGIER_	0.99708	0.42654
YPL061W	ALD6	_SVAVDSSES(ph)NLKK_	2.2932	0.11184

YPL070W	MUK1	_SNS(ph)YDGFR_	1.2592	0.40775
YPL075W	GCR1	_FKLDDKPTPS(ph)QTALDSLLTK_	1.0522	0.46976
YPL075W	GCR1	_KVNEDS(ph)PSSSSK_	0.97108	0.40561
YPL081W	RPS9A	_KAEAS(ph)GEAADEADEADEE_	0.99614	0.42579
YPL085W	SEC16	_SSEEQPENIS(ph)K_	1.9577	0.1692
YPL085W	SEC16	_NSGDDTSM(ox)LFQDDES(ph)DQKVPWEEDVKK_	1.3616	0.35881
YPL085W	SEC16	_KGS(ph)NNSNRPPVIPLGTQEPR_	1.1884	0.44495
YPL085W	SEC16	_SDVPPVSS(ph)STNISPANETQLEIPDTQELHHK_	1.1048	0.49253
YPL085W	SEC16	_AFKPLESDADKYNDVIEDES(ph)DDDNM(ox)STDEAK_	1.0882	0.49699
YPL112C	PEX25	_DDGS(ph)QSPIRK_	1.2545	0.41012
YPL112C	PEX25	_DDGSQS(ph)PIR_	0.98939	0.42038
YPL112C	PEX25	_FSAGSGSESHTESSRS(ph)DDEDSQAK_	0.77174	0.24022
YPL137C	GIP3	_SSS(ph)VSNAALCNTEKPDLK_	0.78238	0.24895
YPL137C	GIP3	_GVAPTAS(ph)KPQT(ph)PILPSPALAVK_	0.58406	0.10174
YPL155C	KIP2	_RSDS(ph)INNNSR_	0.91402	0.35872
YPL169C	MEX67	_AYS(ph)TGAWKTASIAIAQPPQQQASVLPQVASM(ox)NPNITTPPQPQPS(ph)VVPGGMSIPGAPQGAMVM(ox)APT(ph)LQLPPDVQS(ph)R_	2.4324	0.094511
YPL181W	CTI6	_SHAANEAEKS(ph)PR_	1.3888	0.34677
YPL181W	CTI6	_RNS(ph)M(ox)DDASTDQYSLDPGDSDKK_	0.99345	0.42364
YPL190C	NAB3	_GEENGEVINT(ph)EEEEEEEHQQK_	1.0463	0.4652
YPL195W	APL5	_AKNS(ph)PEPNEFLRDQSTDI_	0.97441	0.40831
YPL198W	RPL7B	_ILT(ph)PESQLK_	1.9652	0.16762
YPL217C	BMS1	_IYGKPVQEEDADIDNLPS(ph)DEEPYTNDDDVQDSEPR_	0.75213	0.22426
YPL226W	NEW1	_GTPKPVDT(ph)DDEED_	0.81899	0.27931
YPL228W	CET1	_NLLSNGS(ph)PPM(ox)NDGSDANAK_	0.83647	0.29391
YPL228W	CET1	_ALS(ph)LDDLVNHDENEKVK_	0.55397	0.083931
YPL237W	SUI3	_AGLDNVDAES(ph)K_	1.5151	0.29566
YPL242C	IQG1	_KFTPIEPSLLGPTPS(ph)LEYSPIKNK_	1.5724	0.27499

YPL247C	YPL247C	_RNS(ph)GCDLSATYYASR_	0.99561	0.42537
YPL249C	GYP5	_EVVSS(ph)PENR_	2.8248	0.059515
YPL249C	GYP5	_EVVS(ph)S(ph)PENR_	1.6382	0.25303
YPL260W	YPL260W	_NSS(ph)PAYDEDGNDDRLR_	2.8559	0.057421
YPL263C	KEL3	_SLNGEEDDES(ph)DQDLDEILSSFSKK_	1.0827	0.49291
YPR016C	TIF6	_LQDAQPES(ph)ISGNLR_	1.4707	0.31274
YPR041W	TIF5	_SQNAPSDGTGSST(ph)PQHHDEDEDELSR_	2.1398	0.13496
YPR041W	TIF5	_SQNAPSDGT(ph)GSSTPQHHDEDEDELS(ph)R_	1.969	0.16682
YPR041W	TIF5	_SQNAPSDGT(ph)GS(ph)STPQHHDEDEDELSR_	1.8515	0.1933
YPR041W	TIF5	_SQNAPSDGT(ph)GS(ph)STPQHHDEDEDELSR_	1.7914	0.20848
YPR041W	TIF5	_GGGLS(ph)ISDIAQGK_	1.102	0.4942
YPR041W	TIF5	_AAKPFITWLETAES(ph)DDDEEDDE_	0.87596	0.32698
YPR072W	NOT5	_DVS(ph)ISDR_	1.0672	0.4812
YPR072W	NOT5	_YLM(ox)QPLQEM(ox)S(ph)PK_	0.72368	0.20151
YPR079W	MRL1	_LRS(ph)SPSAS(ph)SSSLANR_	1.0477	0.46631
YPR079W	MRL1	_LRSSPSASSS(ph)SLANR_	0.97937	0.41232
YPR091C	NVJ2	_WYKDNVGNS(ph)SDTEDMDEIDVQDKK_	1.2114	0.43255
YPR091C	NVJ2	_WYKDNVGNSS(ph)DTEDMDEIDVQDKK_	1.0891	0.49765
YPR105C	COG4	_NEDVNIDS(ph)NQSDIET(ph)DGETEK_	1.206	0.43544
YPR108W	RPN7	_VDVEEKSQEVEY(ph)VDPTVNRVPNYEVSEK_	1.98	0.16456
YPR108W	RPN7	_VDVEEKS(ph)QEVEYVDPTVNRVPNYEVSEK_	1.748	0.22021
YPR115W	RGC1	_T(ph)YSAENVPLTSTVSNDK_	1.6334	0.25456
YPR124W	CTR1	_VAIS(ph)ENNQK_	0.6307	0.1323
YPR124W	CTR1	_NNES(ph)KVAISENNQK_	0.62433	0.12793
YPR133C	SPN1	_DRDSS(ph)ATPSSR_	2.6127	0.076246
YPR133C	SPN1	_HISTDFS(ph)DDDLEKEEHNDQSLQPTVENR_	0.99552	0.42529
YPR160W	GPH1	_RLT(ph)GFLPQEIK_	2.7934	0.061719
YPR160W	GPH1	_PPASTSTTNDMITEEPTS(ph)PHQIPR_	2.48	0.089268
YPR160W	GPH1	_PPASTSTTNDM(ox)ITEEPT(ph)SPHQIPR_	2.2622	0.11614

YPR161C	SGV1	_GHIVEKGES(ph)PVVK_	1.2581	0.40827
YPR161C	SGV1	_YT(ph)SVVVTR_	0.92214	0.36546
YPR163C	TIF3	_SGGFGGS(ph)FGGR_	1.1145	0.48677
YPR185W	ATG13	_SPLQPQES(ph)QEDLMDFVK_	1.4105	0.33741
YPR185W	ATG13	_GGGNSS(ph)TSALNSR_	1.1784	0.45043

Table 3.5. Analysis of strain fitness in liquid normal (SC) media.

		OD_{660}					
Strain	**Time (hrs)**	**0**	**2.5**	**5.5**	**8.75**	**12**	**14.5**
825x337		0.183	0.366	1.365	3.015	10.050	13.680
bud6Δ/Δ		0.061	0.225	0.725	2.265	6.450	11.025
hxt1Δ/Δ		0.138	0.235	0.742	2.195	13.100	21.255
itr1Δ/Δ		0.137	0.223	0.569	1.617	8.260	14.925
lrg1Δ/Δ		0.111	0.189	0.660	1.709	12.770	21.450
mds3Δ/Δ		0.129	0.236	0.659	2.093	11.710	16.200
npr3Δ/Δ		0.122	0.233	0.639	1.842	12.090	19.350
pda1Δ/Δ		0.109	0.123	0.301	0.833	2.425	5.120
pdr5Δ/Δ		0.087	0.156	0.358	1.602	4.105	9.440
prb1Δ/Δ		0.113	0.151	0.378	1.526	4.030	9.140
ptr2Δ/Δ		0.111	0.181	0.436	1.406	4.200	9.490
rbs1Δ/Δ		0.117	0.223	0.400	1.740	4.425	9.950
rck2Δ/Δ		0.092	0.247	0.549	2.060	5.335	9.640
scp160Δ/Δ		0.106	0.175	0.453	1.492	4.150	8.790
tpo4Δ/Δ		0.098	0.160	0.364	1.520	5.340	11.280
bud6-S347A		0.120	0.213	0.542	1.729	6.960	10.440
itr1-S26A		0.110	0.266	0.637	1.961	6.150	11.950
lrg1-S605A		0.128	0.215	0.530	1.690	5.855	12.940
npr3-S486A		0.103	0.197	0.498	1.640	5.230	15.600
pda1-Y309A		0.102	0.146	0.364	1.003	2.820	7.710
pda1-S313A		0.112	0.194	0.459	1.528	4.840	11.820
pda1-Y309A-S313A		0.104	0.204	0.537	1.694	5.400	14.400

Table 3.6. Analysis of strain fitness in liquid low nitrogen (SLAD) media.

		OD_{660}					
Strain	**Time (hrs)**	**0**	**2.5**	**5.5**	**8.75**	**12**	**14.5**
825x337		0.149	0.373	1.130	2.199	6.870	7.410
bud6Δ/Δ		0.085	0.199	0.646	1.643	3.100	3.830
hxt1Δ/Δ		0.124	0.207	0.570	1.238	2.650	3.910
itr1Δ/Δ		0.143	0.183	0.429	0.879	1.370	2.490
lrg1Δ/Δ		0.171	0.237	0.544	1.038	3.210	3.140
mds3Δ/Δ		0.134	0.202	0.567	1.331	2.400	3.530
npr3Δ/Δ		0.120	0.171	0.312	0.443	1.370	2.900
pda1Δ/Δ		0.127	0.167	0.210	0.214	0.391	0.485
pdr5Δ/Δ		0.134	0.167	0.397	0.606	0.736	1.069
prb1Δ/Δ		0.109	0.177	0.314	0.531	0.728	0.841
ptr2Δ/Δ		0.114	0.201	0.354	0.586	0.769	1.085
rbs1Δ/Δ		0.111	0.241	0.450	0.690	0.887	1.204
rck2Δ/Δ		0.101	0.265	0.522	0.886	1.688	1.478
scp160Δ/Δ		0.101	0.193	0.435	0.587	0.880	1.140
tpo4Δ/Δ		0.116	0.190	0.395	0.523	0.780	1.006
bud6-S347A		0.130	0.160	0.379	0.652	0.979	1.178
itr1-S26A		0.102	0.170	0.488	0.827	1.301	1.750
lrg1-S605A		0.118	0.199	0.413	0.688	1.027	1.300
npr3-S486A		0.109	0.176	0.378	0.676	0.990	1.038
pda1-Y309A		0.104	0.121	0.221	0.411	0.747	1.115
pda1-S313A		0.130	0.142	0.269	0.563	0.871	1.043
pda1-Y309A-S313A		0.099	0.158	0.335	0.611	1.026	1.385

Table 3.7. Analysis of strain fitness in liquid low nitrogen, low glucose (SLALD) media.

		OD_{660}					
Strain	**Time (hrs)**	**0**	**2.5**	**5.5**	**8.75**	**12**	**14.5**
825x337		0.138	0.348	0.985	1.340	4.000	3.340
bud6Δ/Δ		0.143	0.155	0.552	1.185	2.090	2.520
hxt1Δ/Δ		0.120	0.232	0.559	0.951	1.730	1.730
itr1Δ/Δ		0.109	0.208	0.438	0.645	1.486	1.970
lrg1Δ/Δ		0.124	0.173	0.447	0.710	1.071	1.200
mds3Δ/Δ		0.101	0.206	0.542	0.888	1.853	2.610
npr3Δ/Δ		0.135	0.159	0.284	0.408	0.734	1.100
pda1Δ/Δ		0.097	0.136	0.185	0.236	0.294	0.346
pdr5Δ/Δ		0.093	0.198	0.383	0.560	0.620	0.894
prb1Δ/Δ		0.107	0.203	0.397	0.569	0.687	0.800
ptr2Δ/Δ		0.095	0.158	0.382	0.554	0.699	0.767
rbs1Δ/Δ		0.075	0.215	0.459	0.595	0.719	0.984
rck2Δ/Δ		0.098	0.250	0.461	0.655	0.800	1.012
scp160Δ/Δ		0.105	0.216	0.429	0.528	0.700	0.853
tpo4Δ/Δ		0.112	0.173	0.356	0.539	0.663	0.870
bud6-S347A		0.130	0.137	0.350	0.540	0.747	0.835
itr1-S26A		0.117	0.207	0.375	0.631	0.888	1.010
lrg1-S605A		0.093	0.189	0.310	0.520	0.740	0.850
npr3-S486A		0.128	0.139	0.284	0.460	0.629	0.720
pda1-Y309A		0.107	0.117	0.178	0.256	0.302	0.352
pda1-S313A		0.106	0.161	0.252	0.431	0.607	0.740
pda1-Y309A-S313A		0.103	0.132	0.313	0.440	0.620	0.725

References

1. Lengeler KB et al. (2000) Signal transduction cascades regulating fungal development and virulence. *Microbiology and molecular biology reviews : MMBR* 64:746–85.

2. Fortwendel JR et al. (2012) Plasma membrane localization is required for RasA-mediated polarized morphogenesis and virulence of Aspergillus fumigatus. *Eukaryotic cell* 11:966–77.

3. Karkowska-Kuleta J, Rapala-Kozik M, Kozik A (2009) Fungi pathogenic to humans: molecular bases of virulence of Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus. *Acta biochimica Polonica* 56:211–24.

4. Wang L, Zhai B, Lin X (2012) The Link between Morphotype Transition and Virulence in Cryptococcus neoformans. *PLoS pathogens* 8:e1002765.

5. Lo HJ et al. (1997) Nonfilamentous C. albicans mutants are avirulent. *Cell* 90:939–49.

6. Gale C a et al. (1998) Linkage of adhesion, filamentous growth, and virulence in Candida albicans to a single gene, INT1. *Science (New York, N.Y.)* 279:1355–8.

7. Huang G (2012) Regulation of phenotypic transitions in the fungal pathogen Candida albicans. *Virulence* 3:251–61.

8. Kumamoto CA, Vinces MD (2005) Contributions of hyphae and hypha-co-regulated genes to Candida albicans virulence. *Cellular microbiology* 7:1546–54.

9. Goldstein AL, McCusker JH (2001) Development of Saccharomyces cerevisiae as a model pathogen. A system for the genetic identification of gene products required for survival in the mammalian host environment. *Genetics* 159:499–513.

10. Liu H, Kohler J, Fink GR (1994) Suppression of hyphal formation in Candida albicans by mutation of a STE12 homolog. *Science* 266:1723.

11. Madhani HD, Fink GR (1998) The control of filamentous differentiation and virulence in fungi. *Trends in cell biology* 8:348–53.

12. Berman J, Sudbery PE (2002) Candida Albicans: a molecular revolution built on lessons from budding yeast. *Nature reviews. Genetics* 3:918–30.

13. Gancedo JM (2001) Control of pseudohyphae formation in Saccharomyces cerevisiae. *FEMS microbiology reviews* 25:107–23.

14. Gimeno CJ, Ljungdahl PO, Styles C a, Fink GR (1992) Unipolar cell divisions in the yeast S. cerevisiae lead to filamentous growth: regulation by starvation and RAS. *Cell* 68:1077–90.

15. Liu H, Styles C, Fink G (1993) Elements of the yeast pheromone response pathway required for filamentous growth of diploids. *Science* 262:1741–1744.

16. Roberts RL, Fink GR (1994) Elements of a single MAP kinase cascade in Saccharomyces cerevisiae mediate two developmental programs in the same cell type: mating and invasive growth. *Genes & development* 8:2974–85.

17. Kron SJ, Gow NA (1995) Budding yeast morphogenesis: signalling, cytoskeleton and cell cycle. *Current opinion in cell biology* 7:845–55.

18. Cullen PJ, Sprague GF (2002) The roles of bud-site-selection proteins during haploid invasive growth in yeast. *Molecular biology of the cell* 13:2990–3004.

19. Pan X, Heitman J (2000) Sok2 regulates yeast pseudohyphal differentiation via a transcription factor cascade that regulates cell-cell adhesion. *Molecular and cellular biology* 20:8364–72.

20. Jin R, Dobry CJ, McCown PJ, Kumar A (2008) Large-scale analysis of yeast filamentous growth by systematic gene disruption and overexpression. *Mol Biol Cell* 19:284–296.

21. Ryan O et al. (2012) Global Gene Deletion Analysis Exploring Yeast Filamentous Growth. *Science* 337:1353–1356.

22. Cullen PJ, Sprague GF (2012) The regulation of filamentous growth in yeast. *Genetics* 190:23–49.

23. Saito H (2010) Regulation of cross-talk in yeast MAPK signaling pathways. *Current opinion in microbiology* 13:677–83.

24. Toda T, Cameron S, Sass P, Zoller M, Wigler M (1987) Three different genes in S. cerevisiae encode the catalytic subunits of the cAMP-dependent protein kinase. *Cell* 50:277–87.

25. Robertson LS, Causton HC, Young RA, Fink GR (2000) The yeast A kinases differentially regulate iron uptake and respiratory function. *Proceedings of the National Academy of Sciences of the United States of America* 97:5984–8.

26. Liu H, Styles CA, Fink GR (1996) Saccharomyces cerevisiae S288C has a mutation in FLO8, a gene required for filamentous growth. *Genetics* 144:967–78.

27. Hedbacker K, Carlson M (2008) SNF1/AMPK pathways in yeast. *Frontiers in bioscience : a journal and virtual library* 13:2408–20.

28. Kuchin S, Vyas VK, Carlson M (2002) Snf1 protein kinase and the repressors Nrg1 and Nrg2 regulate FLO11, haploid invasive growth, and diploid pseudohyphal differentiation. *Molecular and cellular biology* 22:3994–4000.

29. Kuchin S, Vyas VK, Carlson M (2003) Role of the yeast Snf1 protein kinase in invasive growth. *Biochemical Society transactions* 31:175–7.

30. Vinod PK, Sengupta N, Bhat PJ, Venkatesh K V (2008) Integration of global signaling pathways, cAMP-PKA, MAPK and TOR in the regulation of FLO11. *PloS one* 3:e1663.

31. Cullen PJ, Sprague GF (2000) Glucose depletion causes haploid invasive growth in yeast. *Proceedings of the National Academy of Sciences of the United States of America* 97:13619–24.

32. Geladé R, Velde S Van de, Dijck P Van, Thevelein JM (2003) Multi-level response of the yeast genome to glucose. *Genome biology* 4:233.

33. Wilson WA, Hawley SA, Hardie DG (1996) Glucose repression/derepression in budding yeast: SNF1 protein kinase is activated by phosphorylation under derepressing conditions, and this correlates with a high AMP:ATP ratio. *Current biology : CB* 6:1426–34.

34. Kraakman L et al. (1999) A Saccharomyces cerevisiae G-protein coupled receptor, Gpr1, is specifically required for glucose activation of the cAMP pathway during the transition to growth on glucose. *Molecular microbiology* 32:1002–12.

35. Zaman S, Lippman SI, Zhao X, Broach JR (2008) How Saccharomyces responds to nutrients. *Annual review of genetics* 42:27–81.

36. Yang Z, Bisson LF (1996) The SKS1 protein kinase is a multicopy suppressor of the snf3 mutation of Saccharomyces cerevisiae. *Yeast (Chichester, England)* 12:1407–19.

37. Vagnoli P, Bisson LF (1998) The SKS1 gene of Saccharomyces cerevisiae is required for long-term adaptation of snf3 null strains to low glucose. *Yeast (Chichester, England)* 14:359–69.

38. Bharucha N et al. (2008) Analysis of the yeast kinome reveals a network of regulated protein localization during filamentous growth. *Mol Biol Cell* 19:2708–2717.

39. Amberg DC, Burke DJ, Strathern JN (2005) *Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor). 2005th Ed.

40. Guthrie C, Fink GR (1991) in *Methods in Enzymology*, pp 3–933.

41. Lorenz MC, Heitman J (1997) Yeast pseudohyphal growth is regulated by GPA2, a G protein alpha homolog. *The EMBO journal* 16:7008–18.

42. Han T-L, Cannon RD, Villas-Bôas SG (2011) The metabolic basis of Candida albicans morphogenesis and quorum sensing. *Fungal genetics and biology : FG & B* 48:747–63.

43. Madhani HD, Fink GR (1997) Combinatorial control required for the specificity of yeast MAPK signaling. *Science (New York, N.Y.)* 275:1314–7.

44. Mumberg D, Müller R, Funk M (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene* 156:119–22.

45. Wach A (1996) PCR-synthesis of marker cassettes with long flanking homology regions for gene disruptions in S. cerevisiae. *Yeast (Chichester, England)* 12:259–65.

46. Baudin A, Ozier-Kalogeropoulos O, Denouel A, Lacroute F, Cullin C (1993) A simple and efficient method for direct gene deletion in Saccharomyces cerevisiae. *Nucleic acids research* 21:3329–30.

47. Longtine MS et al. (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. *Yeast (Chichester, England)* 14:953–61.

48. Zheng L, Baumann U, Reymond J-L (2004) An efficient one-step site-directed and site-saturation mutagenesis protocol. *Nucleic acids research* 32:e115.

49. Gietz RD, Schiestl RH (2007) High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. *Nature protocols* 2:31–4.

50. Xu T et al. (2010) A profile of differentially abundant proteins at the yeast cell periphery during pseudohyphal growth. *The Journal of biological chemistry* 285:15476–88.

51. Prinz S et al. (2004) Control of yeast filamentous-form growth by modules in an integrated molecular network. *Genome research* 14:380–90.

52. Kweon HK, Andrews PC (2013) Quantitative analysis of global phosphorylation changes with high-resolution tandem mass spectrometry and stable isotopic labeling. *Methods (San Diego, Calif.)* 61:251–9.

53. Kweon HK, Håkansson K (2006) Selective zirconium dioxide-based enrichment of phosphorylated peptides for mass spectrometric analysis. *Analytical chemistry* 78:1743–9.

54. Cox J, Mann M (2008) MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nature biotechnology* 26:1367–72.

55. Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M (2012) KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic acids research* 40:D109–14.

56. Zuberi K et al. (2013) GeneMANIA prediction server 2013 update. *Nucleic acids research* 41:W115–22.

57. Chatr-Aryamontri A et al. (2013) The BioGRID interaction database: 2013 update. *Nucleic acids research* 41:D816–23.

58. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif.)* 25:402–8.

59. Cheong H, Klionsky DJ (2008) in *Autophagy: Lower Eukaryotes and Non-Mammalian Systems, Part A* (Academic Press), pp 1–26.

60. Nunnari J, Wong ED, Meeusen S, Wagner JA (2002) in *Guide to Yeast Genetics and Molecular and Cell Biology Part C*, eds Guthrie C, Fink GR (Academic Press), pp 381–393.

61. Walther A, Wendland J (2003) An improved transformation protocol for the human fungal pathogen Candida albicans. *Current genetics* 42:339–43.

62. Zacchi LF, Gomez-Raja J, Davis DA (2010) Mds3 regulates morphogenesis in Candida albicans through the TOR pathway. *Molecular and cellular biology* 30:3695–710.

63. Wu X, Tu BP (2011) Selective regulation of autophagy by the Iml1-Npr2-Npr3 complex in the absence of nitrogen starvation. *Molecular biology of the cell* 22:4124–33.

64. Lorberg A, Schmitz HP, Jacoby JJ, Heinisch JJ (2001) Lrg1p functions as a putative GTPase-activating protein in the Pkc1p-mediated cell integrity pathway in Saccharomyces cerevisiae. *Molecular genetics and genomics : MGG* 266:514–26.

65. Amberg DC, Zahner JE, Mulholland JW, Pringle JR, Botstein D (1997) Aip3p/Bud6p, a yeast actin-interacting protein that is involved in morphogenesis and the selection of bipolar budding sites. *Molecular biology of the cell* 8:729–53.

66. Nikawa J, Hosaka K, Yamashita S (1993) Differential regulation of two myo-inositol transporter genes of Saccharomyces cerevisiae. *Molecular microbiology* 10:955–61.

67. O'Rourke SM, Herskowitz I (1998) The Hog1 MAPK prevents cross talk between the HOG and pheromone response MAPK pathways in Saccharomyces cerevisiae. *Genes & development* 12:2874–86.

68. Pronk JT, Yde Steensma H, Dijken JP Van (1996) Pyruvate metabolism in Saccharomyces cerevisiae. *Yeast (Chichester, England)* 12:1607–33.

69. Wenzel TJ, Berg MA van den, Visser W, Berg JA van den, Steensma HY (1992) Characterization of Saccharomyces cerevisiae mutants lacking the E1 alpha subunit of the pyruvate dehydrogenase complex. *FEBS* 209:697–705.

70. Brown AJ, Gow NA (1999) Regulatory networks controlling Candida albicans morphogenesis. *Trends in microbiology* 7:333–8.

71. Uhl MA, Biery M, Craig N, Johnson AD (2003) Haploinsufficiency-based large-scale forward genetic analysis of filamentous growth in the diploid human fungal pathogen C.albicans. *The EMBO journal* 22:2668–78.

72. Kosako H, Nagano K (2011) Quantitative phosphoproteomics strategies for understanding protein kinase-mediated signal transduction pathways. *Expert review of proteomics* 8:81–94.

73. Macek B, Mann M, Olsen J V (2009) Global and site-specific quantitative phosphoproteomics: principles and applications. *Annual review of pharmacology and toxicology* 49:199–221.

74. Ong S-E (2012) The expanding field of SILAC. *Analytical and bioanalytical chemistry*.

75. Bodenmiller B et al. (2010) Phosphoproteomic Analysis Reveals Interconnected System-Wide Responses to Perturbations of Kinases and Phosphatases in Yeast. *Science Signaling* 3:rs4–rs4.

76. Filiou MD, Martins-de-Souza D, Guest PC, Bahn S, Turck CW (2012) To label or not to label: applications of quantitative proteomics in neuroscience research. *Proteomics* 12:736–47.

77. Chevtzoff C, Vallortigara J, Avéret N, Rigoulet M, Devin A (2005) The yeast cAMP protein kinase Tpk3p is involved in the regulation of mitochondrial enzymatic content during growth. *Biochimica et biophysica acta* 1706:117–25.

78. Rao S et al. (2012) Biogenesis of the preprotein translocase of the outer mitochondrial membrane: protein kinase A phosphorylates the precursor of Tom40 and impairs its import. *Molecular biology of the cell* 23:1618–27.

79. Schmidt O et al. (2011) Regulation of mitochondrial protein import by cytosolic kinases. *Cell* 144:227–39.

80. Reinders J et al. (2007) Profiling phosphoproteins of yeast mitochondria reveals a role of phosphorylation in assembly of the ATP synthase. *Molecular & cellular proteomics : MCP* 6:1896–906.

81. Krause-Buchholz U, Gey U, Wünschmann J, Becker S, Rödel G (2006) YIL042c and YOR090c encode the kinase and phosphatase of the Saccharomyces cerevisiae pyruvate dehydrogenase complex. *FEBS letters* 580:2553–60.

82. Wang Y et al. (2004) Ras and Gpa2 mediate one branch of a redundant glucose signaling pathway in yeast. *PLoS biology* 2:E128.

83. Theodoris G, Bisson LF (2001) DDSE: downstream targets of the SNF3 signal transduction pathway. *FEMS microbiology letters* 197:73–7.

84. Marshall-Carlson L, Neigeborn L, Coons D, Bisson L, Carlson M (1991) Dominant and recessive suppressors that restore glucose transport in a yeast snf3 mutant. *Genetics* 128:505–12.

85. Lorenz MC et al. (2000) The G protein-coupled receptor gpr1 is a nutrient sensor that regulates pseudohyphal differentiation in Saccharomyces cerevisiae. *Genetics* 154:609–22.

86. Pan X, Heitman J (1999) Cyclic AMP-dependent protein kinase regulates pseudohyphal differentiation in Saccharomyces cerevisiae. *Molecular and cellular biology* 19:4874–87.

87. Hogan DA, Sundstrom P (2009) The Ras/cAMP/PKA signaling pathway and virulence in Candida albicans. *Future microbiology* 4:1263–70.

88. Wilson RB, Davis D, Mitchell AP (1999) Rapid hypothesis testing with Candida albicans through gene disruption with short homology regions. *Journal of bacteriology* 181:1868–74.

89. Madhani HD, Styles CA, Fink GR (1997) MAP kinases with distinct inhibitory functions impart signaling specificity during yeast differentiation. *Cell* 91:673–84.