YEAST VOL. **13:** 1099–1133 (1997)

A Review of Phenotypes in *Saccharomyces cerevisiae*

MICHAEL HAMPSEY[1]*

[1]*Department of Biochemistry, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854, U.S.A.*

Received 26 February 1997; accepted 3 April 1997

A summary of previously defined phenotypes in the yeast *Saccharomyces cerevisae* is presented. The purpose of this review is to provide a compendium of phenotypes that can be readily screened to identify pleiotropic phenotypes associated with primary or suppressor mutations. Many of these phenotypes provide a convenient alternative to the primary phenotype for following a gene, or as a marker for cloning a gene by genetic complementation. In many cases a particular phenotype or set of phenotypes can suggest a function for the product of the mutated gene.

Yeast 13: 1099–1133, 1997.
No. of Figures: 0. No. of Tables: 1. No. of References: 204.

KEY WORDS — *Saccharomyces cerevisiae*; functional analysis; phenotypic screening

CONTENTS

*Correspondence to: Michael Hampsey.
Contract grant sponsor: NIH

CCC 0749–503X/97/121099–35 $17.50

INTRODUCTION

Overview

The phenotypes associated with mutations are the most basic tools of genetics. The primary phenotype can be used to genetically follow the mutant allele, to clone the wild-type allele of the primary defect by complementation, or to select for suppressors of the primary defect. For suppressors, a secondary phenotype associated with the suppressor phenotype is often essential for subsequent analyses. Suppressors that do not confer a secondary phenotype, either on their own or in combination with the primary mutation, are usually difficult to define and often not worth pursuing.

Multiple pleiotropic phenotypes associated with single mutations are generally indicative of the importance of the gene product to cell function. Furthermore, certain phenotypes can provide valuable clues to gene function. Presently, less than half of the approximately 6000 genes defined by the *Saccharomyces cerevisiae* genome sequencing project have been identified either genetically or biochemically.[85] Of the remaining genes, only about 30% exhibit sequence similarity to proteins of defined function. This leaves more than a third of all yeast genes for which there is no known function. Furthermore, approximately half of all gene disruptions confer no obvious growth defects. It is therefore imperative to have at hand a repertoire of easily scored phenotypes to screen yeast mutants, which can now be generated at will.

In this review I present a summary of phenotypes compiled from the yeast literature. The emphasis is on phenotypes that can be easily scored or selected. The format of the review is to describe the phenotype, include one or two examples of mutants displaying the phenotype, how it is scored, and, whenever appropriate, discuss functional implications of the phenotype. In all cases, I include at least one reference that describes the phenotype.

I have summarized a broad spectrum of phenotypes. These are described in the text that follows and are summarized in Table 1. Abbreviations are included for phenotypes that are commonly denoted by two- or three-letter symbols. It is important to recognize that this review is by no means comprehensive. Also, it will be rather obvious that the chosen examples are weighted toward my own interest in transcription, reflecting the literature that I know best. I have tried to group specific phenotypes into several general categories. As a cautionary note, though, I want to stress that many of these phenotypes could be included in multiple categories and in many cases a particular phenotype can arise as a consequence of several

Table 1. Summary of phenotypes.

Phenotype[1]	Assay or score[2]	Functional implications[3]	Reference[4]
I. Conditional phenotypes			
Heat-sensitivity (ts)	YPD or SC @ 35°C–38°C	General protein defect; heat-lethality usually indicates an essential gene	12, 181, 188
Cold-sensitivity (cs)	YPD or SC @ 11°C–24°C	General protein defect; cs is often associated with defect in assembly of a multisubunit complex	64, 175, 178
Slow-growth (Slg)	YPD or SC @ 30°C	General protein defect; important for cell growth	129
Ethanol-sensitivity	YPD + 6% ethanol	General protein defect	3
Formamide-sensitivity	YPD + 1·5–3% formamide	General protein defect	2
D_2O-sensitivity	SC made with 90% D_2O	General protein defect	11
II. Cell cycle defects			
G_1 arrest	Small unbudded cells; large unbudded if arrested at Start	Defective in progression through G_1n phase of cell cycle	44, 58, 72, 109
Failure to arrest on G_1	Low proportion of unbudded cells	Failure to arrest at Start in G_1 phase of cell cycle	72, 109, 149
G_2/M arrest	Large budded cells	Defective in progression through the G_2/M transition of cell cycle	72, 109, 149
III. Mating and sporulation defects			
Mating efficiency	Halo formation in response to pheromone-induced growth inhibition	Sometimes correlates with transcriptional defects	72, 153
Sporulation efficiency	Number of asci following induction of sporulation; alternatively, score haploid-specific, drug-resistant segregants	Sometimes correlates with transcriptional defects	72, 153
Inappropriate sporulation	Sporulation in rich medium	Aberrant regulation of entry into meiosis	169
IV. Auxotrophies, carbon catabolite repression and nitrogen utilization defects			
Inositol auxotrophy (Ino)	SD—inositol	Defects in inositol biosynthetic pathway; Ino^- often correlates with transcriptional defects	7, 12
Methionine auxotrophy (Met)	SD—methionine	Defects in methionine biosynthetic pathway; Met^- often correlates with transcriptional defects	105, 125
Phosphate auxotrophy (Pho)	Phosphate-depleted YPD	Defective induction of acid phosphatases; Pho^- often correlates with *PHO5* transcriptional defects	65

Continued

Table 1. Continued

Phenotype[1]	Assay or score[2]	Functional implications[3]	Reference[4]
Sucrose fermentation (Snf; Ssn)	YP + 2% sucrose or rafinose (plus anaerobic conditions for increased stringency)	Defective in carbon catabolite repression; *snf* mutants are generally defective in transcriptional derepression; *ssn* mutants are generally defective in transcriptional repression	25, 132
Maltose fermentation (Mal)	YP + 2% maltose + bromcresol purple	Defective in carbon catabolite repression	88
Galactose fermentation (Gal)	YP + 2% galactose (plus anaerobic conditions for increased stringency)	Often defective in transcriptional activation	87, 88
Respiratory deficiency	YPG (3% glycerol)	Failure to produce respiratory-competent mitochondria	184
Resistance to 2-deoxyglucose	YP + 2% sucrose + 200 µg/ml 2-DG	Constitutive carbon catabolite derepression	133
Accumulation of storage carbohydrates	I_2 staining of glycogen	Defective entry into stationary phase	54, 183
Glutamate auxotrophy	Failure to grow in ammonium medium, rescued by glutamate	TCA and glyoxylate cycle defects; defective retrograde regulation	38, 110, 119
Proline utilization	Failure to grow in proline medium	*PUT* gene defects; ρ^- mutants	18, 19
V. Cell morphology and wall defects			
Flocculence	Ribbon-like colony morphology or clumping in liquid culture	Often associated with transcriptional defects	172
Bud localization	Calcofluor staining; light microscopy	Defects in mechanisms governing bud site selection	30, 148
Elongated cell and bud morphologies	Light microscopy	Sometimes associated with protein phosphatase defects	134, 186
Multibudded cells	Light microscopy	Often associated with defects in progression through G_1 phase of the cell cycle	165, 171
Pseudohyphae formation	Light microscopy; agar 'scarring'	Often associated with defects in MAP kinase signal transduction cascade	60, 152
Osmotic sensitivity (Osm)	YPD + 1·0–1·2 M-sorbitol	Cell wall or cytoskeletal defects	75. 123, 138
Osmotic remediability	YPD + 1·0–1·2 M-sorbitol	Phenotypic suppression of cell lysis, translation, and other defects	123, 148
Calcofluor white	YD + 0·05–0·10% calcofluor white	Defective in cell wall biogenesis	154
Cercosporamide	SC + 5 µg/ml cercosporamide	Defective in cell wall biogenesis	75
Papulacandin B	YD + 20 µg/ml papulacandin B	Defective in cell wall biogenesis	26
Spore wall defects	Sensitivity to 55°C heat shock, exposure to ether, or glusulase	Defective in signal transduction required for spore wall biogenesis	101
Killer toxin: expression, maintenance and resistance	Zone of growth inhibition in response to K^+ strain on methylene blue medium	Defective in many cellular processes, including cell wall biogenesis and secretory pathway	190

VI. Stress response defects			
Sensitivity to heat-shock	Loss of cell viability following 1-h heat shock @ 55°C on SC medium; score survival on YPD medium @ 30°C	Defects in RAS-adenylate cyclase signal transduction pathway	36, 160
Sensitivity to starvation	Loss of cell viability following 2-day incubation on omission medium; score survival on YPD medium	Defects in RAS-adenylate cyclase signal transduction pathway	16, 160
H_2O_2	Zone of growth inhibition surrounding filter disk spotted with 1·5–6 µl of 30% H_2O_2	Altered sensitivity to oxidative stress	99, 100
Menadione	SC + 20–50 µM-menadione	Altered sensitivity to oxidative stress	114
Diamide	SC medium containing 1·5 mM-diamide	Altered sensitivity to oxidative stress	103
Paraquat	SC medium + 1–10 µM-paraquat	Altered sensitivity to oxidative stress	112
Divalent cations and heavy metals	YPD + various concentrations of divalent cations or heavy metals	Altered expression of plasma membrane Altered expression of plasma membrane ATPases; defects in many other biological processes, depending upon the cation or heavy metal used	134, 140, 196
VII. Sensitivity to analogs, antibiotics and other drugs			
Canavanine	SD or -Arg omission medium + 0·8–1·0 µg/ml canavanine	Resistance to low levels of canavanine sometimes correlates with ubiquitin pathway defects	32, 53, 88
Methylamine	See references	Defective ammonium ion uptake	37, 46, 155, 156
D-Histidine	See references	Defective general amino acid uptake	37, 158
3-Aminotriazole	SD + 10–50 mM-3-AT	Induces histidine starvation, invoking general control response; altered sensitivity correlates with general control defects	74, 123
Sulfometuron methyl	SD + 3 µg/ml sulfometuron methyl	Induces isoleucine and valine starvation, invoking general control response; altered sensitivity correlates with general control defects	51
Aminoglycoside antibiotics	Variable	Often correlates with defects in protein synthesis	1, 49, 123
Cycloheximide	YPD + 1 µg/ml cycloheximide	Defects in protein synthesis; cell cycle	124
Trichodermin	YPD + 10 µg/ml trichodermin	Altered peptidyl transferase activity	55
Immunosuppressants	YPD + 0·1 µg/ml rapamycin	Defects in signal transduction (rapamycin); altered amino acid import	68, 69, 163
Oligomycin	YPGE + 1 µg/ml oligomycin	Defective ABC transporter	91
o-Dinitrobenzene	YPD + 175–500 µM-*o*-DNB	Resistance to metals and other toxins	197
Multidrug resistance	Resistance to a broad range of drugs and other toxins; e.g. YPD, pH 4·5, 1 µg/ml reveromycin	Defective ABC transporters; defects in certain gene-specific transcriptional activators	41

Continued

Table 1 Continued

Phenotype[1]	Assay or score[2]	Functional implications[3]	Reference[4]
VIII. Carbohydrate and lipid biosynthesis defects			
Vanadate	YPD + 7–10 mM-*o*-vanadate	Defective protein glycosylation; secretory defects	8, 35
Fenpropimorph	SD + 0·3 μM-fenpropimorph	Defective sterol biosynthesis	104, 120
Nystatin	SD + 1–6 units/ml nystatin	Defective sterol biosynthesis	83
Mevinolin and lovostatin	YPD + 400 μg/ml mevinolin	Defects in sterol biosynthesis	10
IX. Nucleic acid metabolism defects			
UV light	YPD or SC medium exposed to 10–200 Joules/m^2 UV light	Defective repair of UV-induced DNA damage	63, 67, 199
Alkylating agents	YPD + 0·05% MMS	Defective repair of alkylation-induced DNA damage	122
Radiomimetic drugs	YPD + 2–20 μg/ml bleomycin	Defective repair of ionizing- or radiomimetic-induced DNA damage	122
Hydroxyurea	YPD + 100 mM-hydroxyurea	Defective DNA replication	204
Distamycin A	YPD + 80–400 μM-distamycin or YPG + 4–20 μM-distamycin	Defective DNA replication	61
Actinomycin D	SC + 10 μM-actinomycin D	Defective DNA replication	47
Camptothecin	SC + 0·1 μg/ml camptothecin	Defective DNA replication, transcription and recombination due to effects on topoisomerase activity	48, 92
Ciclopyroxolamine	See reference	Defective DNA replication	107
6-Azauracil	SC + 30 μg/ml 6-azauracil	Defective transcription elongation	5, 50
Mycophenolic acid	YPD medium + 45 μg/ml mycophenolic acid	Defective transcription elongation	147
Thiolutin	YPD + 3 μg/ml thiolutin	Defective transcription; defective RNA polymerase II	71
Inositol secretion (Opi)	Crossfeeding of *ino1* mutants on -Ino medium	Defects in transcriptional repression	79, 81, 189
Mutator phenotype	Resistance to α-aminoadipate, cycloheximide, canavanine or 5-fluoro-orotic acid	Enhanced rate of mutations in *LYS2*, *CYH2*, *CAN1* or *URA3*	43
X. A few other phenotypes			
pH-sensitivity	YPD, pH 3·0	Defective vacuole function	9
Sensitivity to benomyl, nocodazole and thiabendazole	YPD + 0·5 μg/ml benomyl (for sensitive mutants)	Defective microtubule function	174
Staurosporine	YPD + 0·1 μg/ml staurosporine	Defective protein kinase C; cell signaling; plasma membrane development	136, 168
Caffeine	YPD + 8–10 mM-caffeine	Defective MAP kinase signaling pathways; other defects	54, 134

[1]Phenotypes with a commonly used two- or three-letter symbol are denoted in parentheses.
[2]A standard method for scoring each phenotype is indicated. More than a single assay or score has been described for most of these phenotypes (see the corresponding sections of the text). Standard media, including YP, YPD, YPG, SC, SD are defined by Sherman.[166]
[3]The functional implications associated with these phenotypes are intended to denote a common functional defect. However, it should be recognized that a broad spectrum of functional defects is often associated with certain phenotypes.
[4]The listed references are taken from the text and are not comprehensive.

distinctly different functional defects. In other words, the principal objective of this review is simply to provide a compendium of phenotypes that have proven useful to the yeast community.

Genetic considerations

Although genetic analysis of yeast mutants is beyond the scope of this review, it is important to stress that genetic linkage between a primary phenotype and any potential pleiotropic phenotype must be established. This is usually done by following phenotypes through meiosis. Consider, for example, the *sua-7-1* mutation, which suppresses the effect of an aberrant ATG start codon in the leader region of the *cyc1-5000* gene.[145] The effect of the *sua7-1* primary mutation in the *cyc1* background is to restore respiratory capacity, scored as growth on lactate medium (Lat phenotype). Thus, the primary phenotype of the *sua7-1* suppressor of *cyc1-5000* is Lat^+. In addition, a *cyc1-5000 sua7-1* mutant is cold-sensitive (cs^-). But is cs^- a pleiotropic phenotype of the *sua7* suppressor? This was determined by a backcross of the *cyc1-5000 sua7-1* revertant (Lat^+ cs^-) with a *cyc1-5000 SUA7*$^+$ mutant (Lat^- cs^+), followed by sporulation and dissection of the resulting diploid. As expected for a single-gene suppressor of *cyc1-5000*, the $Lat^+ : Lat^-$ phenotypes segregated 2 : 2. Similarly, the $cs^+ : cs^-$ phenotypes segregated 2 : 2, demonstrating that the cs^- phenotype is also conferred by a single-gene mutation. Moreover, the Lat^+/cs^- and Lat^-/cs^+ phenotypes cosegrated, thereby confirming that cs^- is indeed a pleiotropic phenotype of the *sua7-1* suppressor. Since cs^-, but not Lat^+, could be counterselected, cs^- was exploited to clone the *SUA7* wild-type gene from a genomic library,[145] and subsequently to select for suppressors of the *sua7-1* defect.[175]

It is also important to determine whether a suppressor phenotype is manifest in the absence of the primary mutation. If this is the cases, then the suppressor phenotype can be followed as a single-gene characteristic, simplifying analysis of the suppressor.[82] Using the example above, the cs phenotype associated with *sua7-1* was found to be independent of the *cyc1-5000* allele. However, a suppressor phenotype that is dependent upon the primary mutation can also be extremely valuable since this establishes a functional (genetic) relationship between the two genes. For example, the *ssu71-1* suppressor of the *sua7-1* cs^- phenotype confers a heat-lethal phenotype, but only in the presence of *sua7-1*; a *SUA7*$^+$ *ssu71-1* mutant has no phenotype. This result provided the first clue that *SSU71* is functionally related to *SUA7*. Subsequent analysis identified *SSU71* (*TFG1*) as the structural gene for the largest subunit of the general transcription factor TFIIF, a satisfying and informative result for a suppressor of the *sua7-1*-encoded form of TFIIB.[175]

A standard procedure to determine whether a secondary phenotype is dependent upon the primary mutation is to examine the meiotic progeny of a diploid resulting from a cross between the suppressor and a wild-type strain. Recovery of the suppressor phenotype in 50% of the progeny establishes that the suppressor phenotype is independent of the primary mutation, whereas a suppressor phenotype that is dependent upon the primary mutation will be recovered in 25% of the progeny. Alternatively, a recessive primary mutation can be complemented by a plasmid-borne wild-type allele and the resulting merodiploid can be scored for the secondary phenotype.

Media

Many of the phenotypes summarized here are scored on standard media, including rich (YPD), glycerol (YPG), synthetic complete (SC), synthetic minimal (SD), and omission (e.g. -Ura) media.[166] Most other media are prepared by addition of the indicated compounds to either YPD or SC medium. In all cases the medium composition is either described or a reference is provided.

CONDITIONAL PHENOTYPES

The concept of conditional mutants was first introduced by Horowitz and Leupold to isolate mutants that are defective in genes essential for cell viability.[76] Accordingly, conditional mutants are those which grow well under permissive conditions, yet are inviable or grow slowly under the restrictive condition. Heat- and cold-sensitivity are the most common conditional phenotypes and are certainly the easiest to score. Nonetheless, the repertoire of conditional phenotypes includes many others. Conditional phenotypes generally refer to sensitivity to the particular condition, although in some cases resistance is the relevant phenotype. It should also be noted that conditional phenotypes can be assessed with respect to specific functions. As an example, *rad55* null mutants are cold-sensitive and osmotic remedial

for repair of ionizing radiation-induced DNA damage.[117]

Heat-sensitivity (ts)

Heat-sensitive mutations are generally indicative of defects in protein coding genes and often define genes that are essential for cell viability. Hartwell exploited the ts phenotype to obtain a collection of yeast mutants that turned out to be extraordinarily valuable for defining genes involved in essential cellular events including replication, transcription, translation, cell cycle control, and formation of the cytoskeleton.[66] Heat-sensitive mutants are defined by distinctly impaired growth at elevated temperature, with little or no growth impairment relative to a related wild-type strain at normal temperature. Heat-sensitivity is typically scored on rich (YPD) medium, although ts is sometimes more pronounced on SC medium. Significant threshold effects are often observed for ts mutants. For example, certain *sua8/rpb1* ts mutants do not form discernible colonies at 38°C, yet grow nearly as well as the parent strain at 36°C.[12]

Heat-sensitive alleles of essential genes can be especially useful for addressing the function of the encoded protein. For example, ts mutants sometimes display terminal phenotypes at specific stages of the cell cycle (see below), thereby demonstrating that the affected gene product is required for progression through the cell cycle. As another example, ts *srb* mutants were used to establish a general requirement for the RNA polymerase II holoenzyme complex *in vivo*.[181] Conversely, ts *taf* mutants were used to demonstrate, quite unexpectedly, that TAF components of the core transcription factor TFIID are not generally required for transcriptional activation *in vivo*.[188]

Cold-sensitivity (cs)

Cold-sensitivity is most often associated with defects in assembly of multisubunit complexes, presumably because protein–protein interactions are entropy driven and intrinsically cs.[161] Accordingly, this phenotype was first exploited to isolate mutants defective in ribosome assembly.[64,178] Analogous to ts mutants, cs mutants are defined by differential growth rates of parent and mutant strains at reduced temperature, while exhibiting comparable growth rates on the same medium at normal temperature. Mutations that confer cs typically do not also confer ts. A drawback to cs mutants is that the normal control strain often grows very slowly at the restrictive temperature. Occasionally more than 2 weeks is required to ascertain differential growth of normal and mutant strains at very low (e.g., 11°C) temperatures. Nonetheless, cs mutants have been extremely valuable for identifying components of multisubunit complexes. As mentioned above, we uncovered the yeast gene encoding TFIIB, a component of the transcription preinitiation complex, based on a cs mutation in *SUA7*.[145] The gene (*SSU71/TFG1* encoding another component of the complex, the largest subunit of TFIIF, was subsequently identified based on suppression of the *sua7* cs defect. The double *sua7 ssu71* mutant was ts, which was then exploited to clone *SSU71*.[175] As for ts, cs is typically scored on rich medium, although in some cases the phenotype is more pronounced on synthetic medium.

A caveat to cs is that *S. cerevisiae trp1* mutants often exhibit a cs growth defect. Therefore, screens for high-copy suppressors of cs phenotypes in a *trp1* background will often pick up *TRP1*, or genes encoding amino acid permeases, including *TAT2*, *BAP1* and *BAP2* (A. Brys, Z.-W. Sun, W. Zehring and M.H., unpublished results). To avoid this problem, work with cs mutants in a *TRP1* wild-type background.

Slow-growth (Slg)

Slow growth is defined simply by impaired growth at normal temperature, usually on rich (YPD) medium. Although not actually a conditional phenotype, Slg^- is a common phenotype that is easy to score, often serving as a valuable marker to follow a gene, to select for suppressors, or to clone by complementation. For example, a mutation in the *SUA5* gene, isolated as a suppressor of an aberrant ATG codon in the *cyc1* leader region, conferred a pronounced Slg^- phenotype, which was exploited to clone *SUA5*.[129]

There are several disadvantages to Slg^- mutants. First, the phenotype is always manifest; unlike conditional mutants, there is no condition under which a Slg^- mutant grows normally. Therefore Slg^- mutants require long incubation periods for either colony formation or to reach a particular cell density. Secondly, pleiotropic phenotypes associated with Slg^- mutations must be distinguished from phenotypes that are simply due to an impaired rate of growth. Thirdly, upon prolonged incubation, Slg^- mutants will 'catch-up' with wild-type strains, eventually forming

colonies of comparable size. Therefore, Slg^- must be scored within an appropriate window of incubation time. Finally, Slg^- mutants are under constant selective pressure to revert. For this reason, care must always be taken to assure that a Slg^- mutant has neither reverted nor is contaminated by Slg^+ revertants that will quickly overtake the mutant population.

Ethanol sensitivity

Yeast mutants that are sensitive to growth on YPD medium in the presence of 6% ethanol have been described.[3] Genetic analysis of these mutants suggested that a large number of genes can be mutated to produce ethanol sensitivity. About one-third of the ethanol-sensitive mutants were also ts, implying that ethanol-sensitivity and ts arise by a common mechanism. Furthermore, there is a correlation between heat shock and ethanol tolerance, and induction of the chaperonin Hsp104 conferred both thermotolerance and ethanol tolerance.[146] Almost none of the ethanol-sensitive mutants described by Aguilera are glycolytic or lipid biosynthetic pathway mutants. Rather, ethanol-sensitivity most likely correlates with mutations that affect protein stability, a premise consistent with the ability of ethanol to disrupt hydrogen bonds.

Formamide sensitivity

Formamide sensitivity was described recently as a novel conditional phenotype in yeast.[2] Like ethanol, formamide is readily taken up by yeast, but offers the advantage of being non-metabolizable. Aguilera defined formamide-sensitivity as impaired growth on YPD medium containing 3% formamide. In that study, ~30% of formamide-sensitive mutants were also ts, suggesting that sensitivity to formamide and ts share a common basis, presumably disruption of hydrogen bonding. Still, ~30% of formamide-sensitive mutants displayed no other phenotype, thereby defining formamide-sensitivity as a novel conditional phenotype. In my laboratory, many of our wild-type strains grow poorly on 3% formamide. However, we often find differential sensitivity of wild type and mutants on YPD medium containing 1·5–2% formamide.

D_2O sensitivity

Bartel and Varshavsky[11] described sensitivity to D_2O as a novel conditional phenotype. The D_2O-sensitive phenotype was defined as impaired growth on minimal medium containing 90% D_2O. The adverse effect of D_2O is presumably a consequence of an isotope effect on protein conformation, either as a component of the intracellular solvent or as an integral component of the protein. D_2O-sensitive mutants were reported to arise at least as frequently as ts mutants and most D_2O-sensitive mutants did not display other conditional phenotypes. The cost of media containing 90% D_2O and deuterium–hydrogen exchange while preparing and storing the media are limitations to the general use of this phenotype.

CELL CYCLE DEFECTS

Mutations that affect progression through the cell cycle are often recognized simply by observing a population of cells under the light microscope. Cell cycle mutants typically arrest at a specific stage of the cell cycle following a shift to non-permissive growth temperatures. Thus, morphological analyses of cells grown at permissive versus non-permissive temperatures can reveal cell cycle mutants. Cell cycle mutants that do not exhibit a conditional growth defect can also be recognized. For example, some cell cycle mutants exhibit a Slg^- phenotype due to impaired progression through a specific stage of the cell cycle. Such mutants can be recognized by a higher than normal fraction of cells displaying a particular cell cycle morphology.

A convenient method to score cell cycle stages under conditions that promote cell cycle arrest has been described.[108] Strains are grown overnight in medium lacking an auxotrophic marker. The missing nutrient is then added to induce cell growth. Following sonication to disperse clumps, cells are observed under a light microscope using a hemacytometer.

A useful depiction of cell morphologies and cytoskeletal rearrangements that occur during progression through the cell cycle are presented elsewhere.[72,109] These are briefly summarized below. More involved techniques can be used to confirm or extend analysis of cell cycle defects. These include (i) cell size distribution by Coulter counter analysis; (ii) Hoechst staining of DNA to visualize nuclei; (iii) flow cytometry analysis of DNA content by fluorescence-activated cell sorting (FACS); and (iv) cell cycle arrest in response to mating pheromone.

G_1 arrest

A normal, asynchronous population of logarithmically growing yeast includes cells at all stages of progression through the cell cycle. This is in contrast to cells that are restricted in progression through G_1. G_1-arrested cells appear as a uniform population of small unbudded cells. Arrest in G_1 restricts passage through Start (commitment to DNA replication) so that haploid G_1-arrested cells contain a 1N DNA content. Accordingly, flow cytometry can be used to define or confirm arrest in G_1 by the appearance of an abnormally high proportion of cells in an asynchronous population with a 1N DNA content. Examples of G_1-arrested strains include *cdc25^{ts}*, *cdc35/cry1^{ts}* and *ras2^{ts}* mutants.[44,58,127] It should be noted that mutants arrested in G_1 at Start form large unbudded cells, sometimes with shmoo, rather than rounded, morphology. G_1 arrest at Start is typified by mutants defective in the G_1 cyclins and p34^{CDC28} protein kinase (reviewed in references 131, 150). In addition to the G_1 phenotypes reviewed here, certain G_1 mutants exhibit a multibudded cell morphology (see below).

Failure to arrest in G_1

Normal strains of *S. cerevisiae* arrest in G_1 as single unbudded cells when the population enters stationary phase or undergoes nutrient starvation. However, mutants have been described that fail to arrest in G_1, defined by a low proportion of unbudded cells and a high proportion of cells with different size buds. For example, *crl* mutants, isolated as cycloheximide-resistant strains that are ts-lethal, fail to arrest in G_1 when grown to stationary phase or when starved for nitrogen.[123] Thus, both G_1 arrest in logarithmically growing cells, and failure to arrest in G_1 in stationary phase or under starvation conditions, are easily scored phenotypes indicative of cell cycle defects.

G_2/M arrest

Failure to progress through the G_2/M transition of the cell cycle results in formation of large budded cells. DNA replication occurs with nuclear migration to the neck of the bud. Consequently, Hoechst staining of G_2/M, blocked cells reveals single nuclei, typically at the neck of the bud, and FACS analysis shows a 2 N-DNA content. Examples of G_2/M arrest are described for *cdc17* and *cdc20* mutants (early G_2), *cdc15* mutants (late G_2),[149] and for *tsm1* and *taf90* heat-sensitive mutants, which encode altered forms of the TFIID subunits yTAFII150 and yTAFII90.[4,188] These TAF mutants are likely to affect transcription of cell cycle-specific genes since mutants defective in RNA polymerase II transcription do not uniformly arrest at any stage of the cell cycle.[105] This is in contrast to conditional mutants that are defective in DNA replication, which typically arrest as large, budded cells at the restrictive temperature.[42]

MATING AND SPORULATION DEFECTS

The ability of haploid cells to mate, and of diploid cells to undergo sporulation, is dependent upon the expression of genes specific for these developmental programs. Therefore, defects in mating and sporulation are sometimes associated with defects in transcription. As examples, mutations in the *SPT3*, *SPT7*, *SPT8* and *SPT15* genes, which are involved in transcription initiation, are associated with both mating and sporulation defects.[57] Although I have focused on *spt* mutants as examples, there is a vast collection of yeast mutants with mating and sporulation defects. Moreover, many of these mutants affect signal transduction pathways and other processes in addition to transcription.

Mating efficiency

Mating efficiency can be conveniently assayed by a plate test. Serial dilutions of mutant and wild-type strains are spotted onto rich medium, crossed to a comparable strain of opposite mating type, incubated on rich medium for 1 day, and replica printed to minimal medium to select for diploids. Mating-defective mutants, when crossed with one another in this assay, produce fewer homozygous diploid colonies than comparable wild-type strains. Mating-defective mutants crossed with a wild-type strain may or may not produce fewer heterozygous diploid strains than wild type. Using this assay, Roberts and Winston reported that crosses of *spt20*Δ × *spt20*Δ mutants produced significantly fewer diploids than did crosses of either *spt20*Δ × *SPT20* or *STP20* × *SPT20*; thus, *SPT20* is required for efficient mating.[153]

A mating factor assay can also be used to screen for mating defects. In this assay, production of mating pheromone causes growth inhibition of a lawn of cells of opposite mating type, resulting in a

halo around cells that produce the pheromone.[72] Pheromone-induced growth inhibition is generally scored using *sst1/bar1* or *sst2* alleles, which render cells super-sensitive to mating factors.[28,29,173] Mutations at the *SST1* locus are mating-type specific, causing *MAT***a** cells to be supersensitive to α factor. On the other hand, mutations at the *SST2* locus confer supersensitivity to the pheromone of opposite mating type for both *MAT***a** and *MAT*α cells. Mutants to be tested, along with wild-type MAT**a** and *MAT*α control strains, are spotted onto a Petri dish that has been seeded with the tester strain. Plates are scored for halo formation following 2–3 days of incubation at 30°C. Whereas the wild-type strain of the same mating type should cause no growth inhibition, the strain of opposite mating type should cause a distinct halo of inhibition. Mutants defective in production of mating factor will diminish halo formation.

Mating defects can also be mating-type specific. For example, *KEX2* encodes a protease required for production of active killer toxin that is also required for proteolytic processing of α factor.[190] Since α factor is produced and secreted by *MAT*α cells to prepare *MAT***a** cells for mating, *kex2* mutants are α-sterile. Also, mutations in the *TUP1* and *SSN6/CYC8* genes, which encode a complex involved in glucose repression,[93] confer multiple phenotypes, including α-sterility.[192]

Sporulation efficiency

Sporulation defects can be assayed simply by counting asci. Diploid strains are inoculated into liquid sporulation medium[166] and incubated with constant agitation. Sporulated cultures are then visualized by light microscopy and quantified with a hemacytometer.[153] Sporulation efficiency can also be scored by a plate assay that takes advantage of drug-resistant markers that are manifest only in haploid cells. Two convenient markers are *can1* and *cyh2*, which confer resistance to canavanine and cycloheximide, respectively. Diploid strains that are heterozygous for *can1* or *cyh2* are sensitive to these drugs, whereas haploid segregants carrying either marker are drug-resistant. Therefore, sporulation efficiency can be deduced from the frequency of drug-resistant haploid segregants.[72]

Inappropriate sporulation

Normal diploid strains are induced to enter meiosis and undergo sporulation in nutrient-deficient medium containing potassium acetate. However, certain mutations cause diploid strains to sporulate in rich medium. For example, heat-sensitive *cdc25* and *cdc35* mutants, which are defective in the RAS-adenylate cyclase signal transduction pathway, undergo sporulation in rich medium at the restrictive temperature.[167,169] Abnormal amino acid metabolism, associated with an *spd1* mutation, has also been reported to induce sporulation in rich medium.[45] Sporulation is readily assayed, as described in the preceding section.

AUXOTROPHIES, CARBON CATABOLITE REPRESSION AND NITROGEN UTILIZATION DEFECTS

Auxotrophies

Specific nutritional auxotrophies are most readily explained by failure to express the genes required for biosyntheses of the particular nutrient. In addition, mutants that are defective in components of the transcriptional apparatus often exhibit specific auxotrophies. General transcriptional defects are sometimes evident at the level of carbon catabolite repression. For example, certain *spt15* mutants, which express altered forms of the TATA-binding protein (TBP), are unable to grow on galactose medium.[6] Other auxotrophies are also commonly associated with general transcription factor defects. A simple screen for potential transcription factor mutants is to score for auxotrophies using a complete set of omission medium, using synthetic complete medium as the control. Three common auxotrophies associated with transcription factor mutants are described here.

Inositol auxotrophy (Ino)

Inositol auxotrophy is often indicative of defects in the general transcriptional apparatus, presumably due to the extreme sensitivity of the *INO1* gene to general transcriptional perturbations.[135] As examples, altered forms of the following proteins are associated with distinct Ino$^-$ phenotypes: subunits of RNA polymerase II;[7,12] TBP, and the Spt7 protein;[6,57] components of the SWI/SNF complex;[126] and the Sub1 and Spt20/Ada5 transcriptional coactivator proteins.[98,153] Consequently, an Ino$^-$ phenotype is often an important clue that a mutant is defective in a component of the RNA polymerase II general transcriptional machinery. Inositol auxotrophy is scored on

synthetic medium lacking inositol. It is important to establish that impaired growth in the absence of inositol is indeed a consequence of inositol limitation, which is done by scoring for the ability of exogenous inositol to rescue the Ino$^-$ phenotype. Inositol omission medium (-Ino) is prepared as described elsewhere; control medium contains 10 mg/l inositol.[166]

Methionine auxotrophy (Met)

Like Ino$^-$, impaired growth in the absence of exogenous methionine is often associated with transcriptional defects. For example, mutation in the *MMS19* gene, which affects both nucleotide excision repair and RNA polymerase II transcription, confers tight methionine auxotrophy.[105] Also, mutation in the *CPF1* gene, which encodes a centromere-binding protein that also functions in transcription, confers methionine auxotrophy.[125] In the case of the *cpf1* mutation, the Met$^-$ pleiotropic phenotype is leaky. In such cases it is important to establish that growth impairment can be rescued by the addition of exogenous methionine to the medium. Methionine auxotrophy is scored on standard -Met omission medium; control medium contains 20 mg/ml methionine.[166]

Phosphate auxotrophy (Pho)

The *PHO* system, initially characterized by Oshima and colleagues,[142] is involved in regulating phosphate metabolism and has provided valuable insight into a mechanism of signal transduction[106] and the role of chromatin structure in transcriptional regulation.[176] The *PHO5* gene encodes the predominant secreted acid phosphatase and is activated in response to phosphate starvation. Pho$^-$ mutants fail to activate *PHO5* transcription and grow poorly on phosphate-depleted medium (Pho$^-$). Mutations affecting either the Pho2 or Pho4 transactivators, or the Pho81 component of the signal transduction pathway, confer a Pho$^-$ phenotype. Recently, we have also identified Pho$^-$ mutants that are defective in a component of the general transcriptional machinery (W.-H. Wu and M.H., unpublished results). Low phosphate medium is prepared by precipitation of inorganic phosphate from YPD medium using magnesium sulfate and concentrated ammonium hydroxide.[65] Alternatively, phosphate depletion can be mimicked by using a temperature-sensitive allele of the *PHO80* gene, which encodes the cyclin component of the Pho80/Pho85 cyclin/cyclin-dependent kinase pair that functions as a negative regulator of *PHO5* transcription.[106,162]

Carbon catabolite repression

Glucose is the preferred carbon source of *S. cerevisiae*. In the presence of glucose, genes involved in utilization of other carbon sources are repressed by a general regulatory system defined as glucose repression or carbon catabolite repression.[86] Mutants that are unable to utilize alternative carbon sources, including galactose, sucrose, raffinose, maltose and others, are either the result of mutations in structural genes encoding enzymatic activities involved in sugar uptake or fermentation, or are a consequence of mutations in regulatory genes required for either derepression or activation of those genes. Summarized in this section are easily scored phenotypes that are often associated with mutants that either fail to overcome glucose repression (defective in derepression) or fail to induce expression (defective in activation) of genes required for growth on carbon sources other than glucose. Also included in this section is a discussion of phenotypes associated with mutants that fail to maintain glucose repression (defective in repression).

Sucrose fermentation (Snf; Ssn)

The ability of yeast to utilize sucrose and raffinose requires invertase, the product of the *SUC2* gene. *SUC2* is repressed in the presence of glucose and derepressed as much as 200-fold in the presence of alternative carbon sources.[56] There is no activation of *SUC2* expression in the presence of sucrose; however, maximal *SUC2* expression requires low levels of glucose.[143] Mutations in genes designated *SNF* were identified by the inability of mutants to derepress *SUC2* in the presence of either sucrose or raffinose.[25] *SNF* genes encode proteins required for derepression of a large number of genes and include the Snf1 protein kinase and components of the Swi/Snf complex, which functions in overcoming the repressive effects of chromatin.[194] An Snf$^-$ phenotype is defined as impaired growth on either sucrose or raffinose medium, with no growth defect on glucose medium. Snf$^-$ phenotypes are generally associated with defects in genes involved in overcoming glucose repression. However, other mutations can also confer Snf phenotypes. For example, *snf3* mutants are defective in expression

of the high-affinity glucose transporter.[27] Raffinose is a poorer substrate than sucrose for invertase; consequently the ability to utilize raffinose is a more stringent indicator of diminished *SUC2* expression.[132]

Growth of yeast strains on carbon sources other than glucose causes derepression of many genes, including genes involved in respiration. Consequently, cells growing on alternative carbon sources such as galactose, raffinose or sucrose acquire more robust respiratory systems than cells growing on glucose. This effect can partially mask the growth defects associated with *snf* mutations. It is therefore best to score *snf* phenotypes under anaerobic conditions. Anaerobic conditions can be attained by addition to the medium of antimycin A (1 μg/ml), an inhibitor of the electron transport chain; by addition of ethidium bromide (20 μg/ml) to promote deletions within the mitochondrial genome; or by incubation of strains in a GasPak (Difco Laboratories) anaerobic chamber.

Maltose fermentation (Mal)

The *S. cerevisiae* genome contains five *MAL* loci that confer the ability to ferment maltose. Each locus is composed of three genes that code for a maltose permease, maltase and a transcriptional activator.[95] As for other genes involved in alternative carbon source utilization, the *MAL* genes are subject to glucose repression. In the absence of glucose and presence of maltose, expression of the *MAL* genes is induced by the Mal transcriptional activator protein. In this sense, *MAL* gene expression is regulated similarly to *GAL* gene expression (below), but different from *SUC* gene expression (above). The ability of yeast to ferment maltose can be conveniently assayed on indicator medium that includes 2% maltose and bromocresol purple.[88] Maltose-fermenting strains turn yellow on this medium, whereas strains that are unable to ferment maltose remain white.

Galactose fermentation (Gal)

In contrast to utilization of sucrose, fermentation of galactose requires activation rather than derepression of gene expression. In the presence of galactose and absence of glucose, the Gal4 activator induces *GAL* gene expression as much as 1000-fold.[87] Consequently, one class of Gal^- mutants fails to respond to the Gal4 transcriptional activator. An example of the utility of this phenotype is described by Arndt and Winston, who used Gal^- (along with Ino^-) to screen for activation-defective TBP mutants.[6] A Gal^- phenotype is defined by impaired growth on galactose medium with no growth defect on glucose medium. As described for Snf mutants, growth of Gal mutants on galactose medium can be affected by the respiratory capacity of the cell. Thus, the Gal phenotype is best scored under anaerobic conditions (see above).

The Gal^- phenotype can also be scored on galactose indicator medium. In this case the indicator bromthymol blue is added to YPGal medium at 4 mg/ml.[88] Gal^+ strains turn yellow on indicator plates, whereas Gal^- strains remain white. It should be noted that laboratory strains related to S288C are generally *gal2*$^-$. The *GAL2* gene encodes the galactose transporter; consequently, these strains are phenotypically Gal^-. This problem can be circumvented by conversion of strains to *GAL2*$^+$ using plasmid pAA1.[195]

Respiratory deficiency

Respiration-deficient yeast mutants form smaller (petite) colonies on glucose medium as a consequence of the inability to metabolize the ethanol produced by fermentation of glucose.[184] Most petite mutants result from either complete loss of the mitochondrial genome (ρ^0) or from large deletions (ρ^-). The other class of petite mutants are due to mutations in nuclear genes, denoted *PET*, that are required for respiration.

Respiratory-deficient mutants can be recognized by their inability to grow on non-fermentable carbon sources, while retaining the ability to grow on glucose medium. Mitochondrial ρ^- mutants can be distinguished from nuclear *pet* mutants by crossing petite mutants with a ρ^0 *PET*$^+$ tester strain. If the resulting diploid strains grow on a non-fermentable carbon source, the mutants are usually the result of a recessive *pet* mutation. However, some *pet* mutants (e.g., *pet18*) tend to spontaneously become ρ^- or ρ^0, which would lead to misdiagnosis of a *pet* mutant as a ρ mutant. A more definitive distinction is to score the meiotic progeny of a cross between a petite and normal strain. Two : two segregation of the petite phenotype would confirm a single-gene, nuclear defect. The simplest medium to score for respiratory mutants contains 3% glycerol (YPG) as the sole carbon source. Other non-fermentable carbon sources, including ethanol and lactate, are also commonly used and generally provide a more

stringent score for respiratory deficiency. Inability to grow on acetate is diagnostic for a defect in the TCA cycle.[96,110]

Resistance to 2-deoxyglucose

Mutants that are constitutively glucose derepressed have been described. For example, the *ssn* class of mutants were isolated as suppressors of *snf* mutations by selecting for restoration of growth on sucrose medium. An example is the *ssn6/cyc8* suppressor, which confers constitutive *SUC2* expression.[164] Another phenotype associated with constitutive glucose derepression is the ability to grow on sucrose in the presence of 2-deoxyglucose (2-DG). 2-DG is a glucose analog that confers glucose repression, yet is not metabolized. Therefore only strains that are glucose-derepressed can utilize sucrose in the presence of 2-DG.[54,133] 2-DG-resistance is assayed on either rich or synthetic medium containing 2% sucrose and 200 μg/ml 2-DG under anaerobic conditions (GasPak; Difco Laboratories). Sucrose, rather than raffinose, is used as the carbon source to reduce the stringency of the screen.[133]

Accumulation of storage carbohydrates

Glycogen is a storage carbohydrate that accumulates in *S. cerevisiae* under starvation conditions or when cells enter stationary phase.[111] Accordingly, failure to accumulate glycogen is indicative of defective entry into stationary phase. Accumulation of glycogen is conveniently assayed by a simple iodine-staining reaction, which is based on intercalation of I_2 into the tightly coiled helical structure of glycogen.[34] Strains are first grown as either colonies or patches on YPD medium. Plates are then flooded with 0·2% I_2–0·4% KI solution, or by inverting plates over iodine crystals.[24] Strains will stain dark brown or violet in proportion to their intracellular levels of glycogen; glycogen-deficient mutants either do not stain or stain yellow. Glycogen-deficient *glc* mutants include mutations in the *GLC2/SNF1*, *GLC5/RAS2* and *GLC3* genes.[24] Mutations in the *GLC3*-encoded glycogen debranching enzyme change the color of the iodine stain from brown to bluish-purple.[157] *bcy1* and *reg1* mutants are good controls for iodine staining since *bcy1* mutants fail to accumulate glycogen,[23] whereas *reg1* null mutants overaccumulate glycogen.[77]

Nitrogen utilization

Glutamate, asparagine and ammonia are preferred nitrogen sources for yeast.[38,119] Ammonia is assimilated exclusively by its incorporation into glutamate and glutamine. Glutamate dehydrogenase converts ammonia and α-ketoglutarate to glutamate, whereas glutamine synthetase converts ammonia and glutamate to glutamine. *S. cerevisiae* can also use alternative nitrogen sources, including arginine, proline, allantoin, γ-aminobutyrate and urea, when preferred nitrogen sources are unavailable. Genes encoding catabolic enzymes and permeases required for utilization of less preferred nitrogen sources are repressed in the presence of preferred nitrogen sources, a process defined as 'nitrogen repression'. Nitrogen repression is manifest primarily by the products of the *GLN3* and *URE2* genes. Gln3 activates transcription in the absence of preferred nitrogen sources, whereas *URE2* represses transcription when preferred nitrogen sources are available. In this section I summarize a few of the many auxotrophic phenotypes associated with defects in nitrogen regulation.

Glutamate auxotrophy

Glutamate auxotrophy is defined by the inability of cells to grow on medium containing ammonia as the sole nitrogen source, while retaining the ability to grow in the presence of glutamate. Glutamate is synthesized by either glutamate dehydrogenase or glutamate synthase, both of which utilize α-ketoglutarate as a substrate. Consequently, glutamate auxotrophy occurs when both the TCA and glyoxylate cycles are defective. For example, mutations in the *CIT1* and *CIT2* genes, which encode mitochondrial and peroxisomal citrate synthase, respectively, confer glutamate auxotrophy.[96] Mutations in the *RTG1* or *RTG2* genes, which are involved in communication from the mitochondrion to the nucleus (retrograde regulation), also confer glutamate auxotrophy.[110] Glutamate auxotrophy is scored on YNB medium containing 2% glucose in the absence or presence of 0·02% glutamine.[110]

Proline utilization

Cellular nitrogen requirements can be obtained from proline in the absence of preferred nitrogen sources. Proline is converted to glutamate by the reverse of its biosynthetic pathway, although the reactions are catalysed by different enzymes.

Screens for mutants that affect proline utilization identified the *PUT* genes.[17,18,121] *PUT1* and *PUT2* encode the two enzymes required for conversion of proline to glutamate, *PUT3* encodes a transcriptional activator of the proline utilization pathway, and *PUT4* encodes a proline permease. In addition to *put* mutants, ρ^- strains are unable to utilize proline as the sole nitrogen source due to mitochondrial sequestration of the proline catabolic enzymes.[19] Proline utilization is scored in the presence of 0·1% proline as described previously.[17,198]

CELL MORPHOLOGY AND WALL DEFECTS

Flocculence

Cell flocculence is readily scored as severe cell clumping in liquid culture and can usually be seen as a rough or ribbon-like colony morphology on agar plates. Many defects in the RNA polymerase II transcriptional machinery confer cell flocculence. For example, all *ssn* mutations cause severe flocculence.[172] Flocculence can serve not only as a marker in genetic crosses, but has been used successfully as a cloning marker by scoring transformants for restoration of smooth colony morphology.

Bud localization

The surface expansion associated with cell growth in *S. cerevisiae* is normally focused at the bud site.[159] Diploid cells exhibit a bipolar pattern of bud formation, meaning that daughter cells emerge at the pole opposite the bud site from the previous cell cycle. By contrast, haploid cells display an axial bud pattern with the daughter cell emerging adjacent to the previous bud site. The distinction between these two patterns is governed by the mating type locus. Yeast mutants have been identified that alter the normal budding pattern of haploid and/or diploid cells.[30] As examples, mutations in the *BUD1*, *BUD2* or *BUD5* genes result in a random bud pattern in diploid cells and in haploid cells of either mating type, whereas mutations in *BUD3* and *BUD4* specifically affect the axial bud pattern in haploid cells. Mutants displaying altered patterns of bud localization can be recognized by observing cells grown on agar under a light microscope[30] or by staining bud scars with Calcofluor and observing by fluorescence microscopy.[148]

Elongated cell and bud morphologies

Conditional yeast mutants that are defective in cytokinesis become elongated and multinucleate under the restrictive condition. As examples, deletion of either *TPD3* or *CDC55*, which encode homologs of the A and B regulatory subunits, respectively, or mammalian protein phosphatase 2A, caused elongated and multinucleated cells at the restrictive temperature.[186] Overexpression of the *PPH21*-encoded catalytic subunit of protein phosphatase 2A also confers elongated and multinucleate cell morphology. Mutants have also been described that display normal cell morphology, but acquire an elongated bud morphology. Examples are the septin mutants encoded by the *cdc3*, *cdc10*, *cdc11* and *cdc12* alleles.[94] Elongated cell and bud morphologies can be scored by standard microscopy.

Multibudded cells

Cell cycle mutants that are defective in progression through the G_1 phase of the cell cycle sometimes exhibit a multibudded cell morphology. Bud formation depends upon activation of the Cdc28 protein kinase by the G_1 cyclins, Cln1–Cln3. DNA replication also requires activation of Cdc28, in this case by the B-type cyclins, Clb1–Clb6. Mutations in the *CDC34*-encoded ubiquitin conjugating enzyme, as well as mutations in *CLB1–CLB6*, have been shown to result in G_1 arrest with accumulation of multibudded cells.[165] Mutations in the check-point control gene *CDC4* also arrest as multibudded cells.[171] Interestingly, certain transcription factor mutants, including those expressing specific forms of TBP, exhibit growth arrest as multibudded cells at the restrictive growth temperature, comparable to *cdc4* mutants.[39] Multibudded cells can be scored by standard microscopy.

Pseudohyphae formation

S. cerevisiae is dimorphic, existing either in a spherical, unicellular yeast-like morphology or in a filamentous form, termed pseudohyphae, that results from elongated chains of cells that remain attached to one another.[60] The dimorphic transition to pseudohyphal growth is a diploid-specific event that occurs in response to nitrogen starvation. Filamentous growth is controlled by components in the mitogen-activated protein (MAP) kinase signal transduction cascade (*STE20*, *STE11*, *STE7* and *STE12*) that are also components of the pheromone response pathway.[113] Other genes, including *ELM*s (elongated morphology,[13,14] *PHD1* (pseudohyphal growth),[59] and

SHR3 (super high histidine resistant),[115] also affect pseudohyphal growth. Haploid cells can also undergo pseudohyphal growth, a transition that involves a switch from axial to bipolar bud site selection and requires the same MAP kinase components necessary for pseudohyphal growth in diploids.[152] The distinction between yeast-like and filamentous growth is readily apparent using a light microscope: unicellular growth results in a smooth colony morphology, whereas filamentous growth produces colonies with rough edges representing pseudohyphae. Filamentous growth also results in agar penetration. Therefore, this phenotype can be scored as 'scarred' agar after washing cells from the agar surface of a YPD plate.[152]

Osmotic sensitivity (Osm)

Growth impairment under conditions of high osmotic strength is often associated with defects in the cell wall or components of the cytoskeleton,[75,89,138] although other classes of mutants also exhibit osmotic sensitivity, including those affecting vacuolar development[9] and translational fidelity.[123] A MAP kinase signal transduction pathway that involves osmosensing has also been identified.[20] Osmotic sensitivity is typically scored on rich medium containing KCl (0·75–1·5 M), NaCl (0·9–2·5 M), sorbitol (1·0–1·2 M) or glycerol (1·0–2·5 M). Optimal concentrations of these compounds vary, depending on the strain background.

Osmotic remediability

High osmolarity sometimes remediates other phenotypes. This phenomenon is known as phenotypic suppression, since the suppressed phenotype requires the continued presence of the condition (high osmolarity) rather than a genetic condition. There are many examples of osmotic remediability in yeast. In some cases, high osmolarity affects specific cell functions. One well-documented example is suppression of mutations that affect the fidelity of translation elongation by high concentrations of KCl or glycerol.[123] In other cases, high osmolarity suppresses cell lysis defects; for example, 1 M-sorbitol suppresses the cell lysis phenotype of null mutations in components of the MAP kinase pathway.[134]

Calcofluor white

Calcofluor white is a fluorochrome that exhibits antifungal activity and has high affinity for yeast cell wall chitin.[154] Resistance to calcofluor can be used to screen for mutants that are defective in chitin biosynthesis and cell wall morphogenesis.[154] Also, hypersensitivity to calcofluor has been found as a pleiotropic phenotype associated with certain yeast cell wall mutants. In the case of the *cwh47-1* mutant, calcofluor hypersensitivity was exploited to clone *CWH41/PTC1*, the structural gene encoding a type 2C serine/threonine phosphatase.[84] Calcofluor-resistant mutants can be selected on YD medium containing 0·05–0·10% calcofluor white.[154]

Cercosporamide

Cercosporamide is an antifungal antibiotic. Enhanced sensitivity to cercosporamide has been reported for cell wall mutants of yeast. For example, *knr4* mutants, which contain reduced levels of both (1→3)-β-glucan synthase activity and (1→3)-β-glucan content in the cell wall, are cercosporamide sensitive.[75] Sensitivity is scored on synthetic medium containing 5 μg/ml of cercosporamide.[75]

Papulacandin B

Papulacandin B is an antifungal agent that interferes with synthesis of the (1→3)-β-glucan component of the yeast cell wall. Resistance to papulacandin B has been used to select mutants in both *Schizosaccharomyces pombe* and *S. cerevisieae*.[26] A single complementation group was defined in *S. cerevisiae* and designated *pbr1*. The *PBR1* gene encodes a protein that appears to be a component of the (1→3)-β-glucan synthase complex. *PBR1* was also identified in several other genetic selections, including FK506- and cyclosporin A-hypersensitivity (*FKS1*); hypersensitivity to calcofluor white (*CWH53*); resistance to echinocandin (*ETG1*); and synthetic lethality with calcineurin mutations (*CND1*) (reviewed in reference 26). Resistance is scored on YD medium (1% yeast extract, 2% glucose) containing 20 μg/ml of papulacandin B.[26]

Spore wall defects

Yeast spores are normally resistant to heat shock, ether and glusulase digestion. Mutants that are able to complete meiosis but are defective in spore wall formation exhibit enhanced sensitivities to all three of these conditions. For example, a mutation in the *SMK1* gene, which encodes a developmentally regulated MAP kinase required for spore wall assembly, results in a dramatically

reduced plating efficiency following 40-min exposure to 55°C heat shock, 5-min exposure to ether, or 1-h treatment with glusulase.[101] Defects in spore wall formation can also be visualized by phase contrast and fluorescence microscopy.[101]

Killer toxin: expression, maintenance and resistance

Killer strains of *S. cerevisiae* secrete a protein that kills sensitive strains.[190] The killer phenotype (K^+) is encoded by one of several distinct, virus-encapsidated double-stranded RNA molecules. Altered ability of K^+ cells to kill sensitive strains (R^-), or of R^+ strains to resist killing, can arise as a consequence of mutations in different classes of genes, including *MAK* (maintenance of killer), *SKI* (superkiller), *KEX* (killer expression), *REX* (resistance expression) and *SEC* (secretion).[190] Therefore, mutants that are defective in expression or maintenance of the viral genome encoding killer toxin, or in resistance to killer toxin, are suggestive of alterations in a number of basic cellular processes. Prominent among genes associated with killer expression and maintenance are those involved in translation, including genes affecting 60S subunit biogenesis, ribosomal frameshifting, and translation of non-poly(A) mRNA.[190]

Resistance to killer toxin is typically associated with defects in genes involved in the structure or biosynthesis of the cell wall. This is because killer toxin binds to β-glucan components of the wall. For example, the K1 toxin binds (1→6)-β-glucan as the initial step in the action of the toxin.[80] One class of killer-resistant genes are designated *KRE*, for killer resistance.[22] Genetically related genes involved in (1→6)-β-glucan biosynthesis apparently function along a secretory pathway. Consequently, killer-resistant mutants have been valuable for defining secretory pathways,[26] and Golgi components involved in glycosylation of cell wall mannoproteins.[118]

Sensitivity to killer is scored on MB medium, defined as YPD that has been buffered to pH 4·7 with 50 mM-sodium citrate and containing 0·003% methylene blue.[191] Strains to be tested are replica printed from YPD medium to MB medium that has been spread with a lawn of a killer-sensitive strain and incubated at 20°C. Potential K^+ strains should be scored for killer using diploid R^- strains to ensure that the zone of growth inhibition does not correspond to pheromone arrest.

STRESS RESPONSE DEFECTS

Sensitivity to heat shock

Altered sensitivity to heat shock is defined as the ability of cells to survive a brief incubation at high temperature. In contrast to heat-sensitivity (ts; see above), resistance to heat shock is scored at normal growth temperature. In one study, resistance to heat shock was scored by replica printing cells to minimal medium preheated to 55°C, followed by incubation at 55°C for 1 h.[160] Heat-shock sensitivity was then scored as the density of cell patches after 2 days of incubation at 30°C. Resistance to heat shock was used to clone the *PDE2* gene as a dosage-dependent suppressor of heat-shock sensitivity associated with the $RAS2^{val19}$ mutation.[160] Variations of this phenotype have been described. For example, scoring cell viability following prolonged incubation at 39°C and 42°C was used to establish that the Rpb4 subunit of RNA polymerase II is involved in stress tolerance.[36]

Sensitivity to starvation

Sensitivity to nitrogen starvation is another method to score stress tolerance. Sensitivity to nitrogen starvation and heat shock (preceding section) are both hallmarks of defects in the Ras–adenylate cyclase pathway. For example, the $RAS2^{val19}$ allele increases the rate of signaling through the Ras pathway and renders cells sensitive to both conditions.[160] Conversely, high copy expression of *PDE2*, which encodes phosphodiesterase and thereby diminishes signaling through the Ras pathway, suppresses the sensitivity to nitrogen starvation and heat shock associated with $RAS2^{val19}$.[160] Sensitivity to starvation is scored at 30°C by growing cells for 2 days on omission medium, replica printing to synthetic medium lacking nitrogen, incubating for 7 days, replica printing back to rich medium, and scoring for growth following 2 days of incubation.

Sensitivity to starvation can also be scored by a colorimetric assay. In this case, cells are incubated on glucose-limited medium in the presence of erythrosin B, which penetrates and accumulates in dead cells.[16] Consequently, starvation-sensitive mutants turn pink or dark pink on this medium, whereas normal strains remain white. Erythrosin B staining is done on SD medium containing 7·5 μM-erythrosin B.[16]

H_2O_2

Many different enzymes are involved in protecting aerobic cells from the potentially harmful effects of oxygen derivatives. An approach to identifying genes involved in relief of oxygen stress is to screen for mutants that have become sensitive to hydrogen peroxide. In one study, mutants representing 16 complementation groups (*pos* genes) were identified based on enhanced hydrogen peroxide sensitivity.[99] The *pos10* gene is allelic to *ZWF1/MET19*, the gene encoding glucose-6-phosphate dehydrogenase, an enzyme known to be involved in relief of oxidative stress. Interestingly, *pos9* is allelic to *SKN7*, a homolog of prokaryotic 'two-component' response regulators. This result suggests that a two-component system is involved in the oxidative-stress response in yeast.[100]

Sensitivity to hydrogen peroxide can be scored in a simple zone inhibition assay. Cells from liquid culture are streaked radially on a YPD plate with 1·5–6 µl of 30% H_2O_2 spotted onto a filter paper disk. The zone of growth inhibition reflects the degree of sensitivity to hydrogen peroxide.[99] As an alternative to H_2O_2, methylviologen can be used as the oxidant.[100]

Menadione

Menadione (vitamin K_3) is a pro-oxidant that generates superoxide anion ($O_2^{\cdot -}$) through redox cycling.[114] As such, menadione can be used as an inducer of oxidative stress. Deletion of the superoxide dismutase gene (*SOD1*) rendered cells sensitive to menadione-induced oxidative stress, defined by failure of cells to grow on synthetic medium in the presence of menadione. Expression of *CUP1*, which encodes metallothionine, suppresses this effect. Consequently, sensitivity (or resistance) to menadione can be used to score for mutants with altered sensitivity to oxidative stress. Menadione medium is prepared by adding 50 mM-menadione in ethanol to synthetic medium at a final concentration of either 20 µM or 50 µM.[114]

Diamide

Diamide is a thiol-oxidizing drug that induces oxidative stress by depleting cells of reduced glutathione.[103] Therefore, sensitivity to diamide can be used to score for mutants with increased tolerance or resistance to oxidative stress due to changes in glutathione concentrations. In one study, this phenotype was used to clone *Arabidopsis* cDNA that confers diamide tolerance to *S. cerevisiae*. Diamide tolerance is scored on SC medium containing 1·5 mM-diamide.[103]

Paraquat

Paraquat is a generator of superoxide anions. Yeast mutants that are hypersensitive to paraquat have been described. In one study, mutation in the *ATX1* gene, which encodes a small protein with structural similarity to bacterial metal transporters, conferred paraquat hypersensitivity, as well as increased sensitivity to hydrogen peroxide.[112] Apparently *ATX1* protects cells against the toxicity of both superoxide anion and hydrogen peroxide. Paraquat medium consists of SC medium supplemented with 1–10 µM-paraquat.[112]

Divalent cations and heavy metals

Resistance or sensitivity to divalent cations and toxic heavy metals has been extensively studied in yeast. In many cases resistance is conferred by membrane ATPases that serve to pump toxins from the cell; in other cases oxidoreductases are responsible for detoxification. I have included here the effects of only two cations, Ca^{2+}, a divalent cation that is essential for cell growth, and Cd^{2+}, a cell toxin. Phenotypes associated with high levels of other elements, including arsenite, cobalt, chromium, copper, iron, mercury, magnesium, manganese, nickel, lead and zinc, have also been described.[32,197] Be careful to distinguish between a cation-specific effect and an osmotic effect due to high salt concentration. This can be done simply by asking if either sorbitol or KCl is able to confer a similar phenotype.

Ca^{2+} ions affect many cellular processes.[139] Both calcium-sensitive and calcium-dependent yeast mutants have been described. For example, a *cls4* mutant of *S. cerevisiae* failed to grow in the presence of 100 mM-$CaCl_2$, producing large, round, unbudded cells. The *cls4* mutation is allelic to *CDC24*. Characterization of this mutant suggested that the Ca^{2+} ion controls bud formation and bud-localized cell surface growth.[141] Conversely, a *cal1-1* mutant exhibited Ca^{2+}-dependent growth. In Ca^{2+}-poor medium, *cal1* mutants arrested with tiny buds and the nucleus was arrested in the G_2 stage of the cell cycle. In this case the calmodulin inhibitor trifluoperazine could restore growth in Ca^{2+}-poor medium. These results suggested that Ca^{2+} ions and calmodulin play

important roles in the yeast cell division cycle at the stage of bud growth and nuclear division.[140] Phenotypic suppression by elevated levels of Ca^{2+} has also been reported. For example, the ts effect of *bck1* null mutations, affecting a MAP kinase pathway, is suppressed by addition of 25 mM-Ca^{2+} to the medium.[134] Neither 50 mM-KCl nor 75 mM-sorbitol had the same suppressive effect, demonstrating that the effect is due specifically to Ca^{2+} and not to osmotic remediation.

Yeast cell growth is inhibited by low levels of cadmium. The toxicity associated with cadmium might be due, in part, to disruption of protein structure.[32] There appear to be multiple mechanisms by which yeast resist the toxic effects of cadmium. One factor involved in resistance is glutathione, which binds heavy metals and is also involved in the detoxification of reactive oxygen intermediates.[32] Mutations in the *YAP1* gene, which encodes a homolog of the mammalian transcription factor c-Jun, confer cadmium hypersensitivity, and overproduction of either *YAP1* or *CAD1*, another c-Jun homolog, confers multidrug resistance and tolerance to toxic levels of cadmium, zinc, and the iron chelator, 1,10-phenanthroline.[196] Cadmium sensitivity can be scored on YPD medium containing 5–10 mg/l of cadmium chloride.[32]

SENSITIVITY TO ANALOGS, ANTIBIOTICS AND OTHER DRUGS

There is a plethora of amino acid analogs, many of which have been useful for identifying and characterizing transport systems, general amino acid control, and amino acid biosynthetic pathways in *S. cerevisiae*. An excellent summary of these analogs was published by Cooper.[37] Only a few of these analogs and associated phenotypes are reviewed here.

This section also includes antibiotics. Altered sensitivities to many of these drugs have been instrumental in defining components and functions associated with the translational machinery. Accordingly, resistance or sensitivity is often indicative of translational defects. As for the amino acid analogs, there is an enormous number of these compounds, only a few of which are described below. Finally, this section includes several other drugs and toxins that can be useful for identifying defects associated with transporters and signal transduction pathways.

Canavanine

Canavanine is an arginine analog that is imported into yeast cells by the *CAN1*-encoded arginine permease. Canavanine is readily incorporated into proteins *in vivo*, resulting in accumulation of aberrant proteins. Resistance to high levels of canavanine (60 mg/l) is conferred exclusively by mutations at the *can1* locus. However, low-level canavanine resistance (e.g., 0·8 mg/l) can arise by mutations at other loci or by overexpression of ubiquitin. Aberrant proteins containing amino acid analogs are degraded by the ubiquitin pathway,[53] and overexpression of ubiquitin can suppress canavanine toxicity.[32] These results suggest that low-level canavanine resistance or sensitivity might correlate with alterations in the ubiquitin pathway of protein turnover. Since canavanine is a competitive inhibitor of arginine, arginine must be excluded from the media used to score for canavanine sensitivity. Also, canavanine sensitivity must be scored in the presence of preferred nitrogen sources (e.g., SD or -Arg medium) to prevent induction of the general amino acid permease system that provides an alternative route for uptake of arginine and canavanine.[88] Thus, canavanine medium typically consists of SD or -Arg omission medium containing variable concentrations of canavanine.

Sensitivity of yeast mutants to many other analogs has been described. For example, the *crl* mutants were isolated as cycloheximide-resistant strains that are heat-lethal and hypersensitive to the alanine analogs β-2-thienylalanine, β-chloroalanine and triazolealanine.[123] Some *crl* mutants are also hypersensitive to 3-aminotriazole (3-AT), suggesting that these *crl* mutants fail to invoke the general control response. Analogs that have been useful for defining transport systems, and their resistance phenotypes, are reviewed by Cooper.[37]

Methylamine

Methylamine is an ammonia analog, whose uptake is mediated by an active transport system. Three genes, designated *amt*, *mep1* and *mep2*, were identified based on their ability to confer methylamine resistance and are required for either high- or low-capacity ammonia import.[46,156] Thus, methylamine resistance correlates with defects in the ammonia transport system. Methylamine resistance must be scored in the absence of preferred nitrogen sources (glutamine, asparagine and

ammonia) on medium described by Larimore and colleagues.[155]

D-Histidine

The *GAP* genes encode components of the general amino acid transport system. Mutations in *GAP* genes can be directly selected on proline medium in the presence of D-histidine.[158] Accordingly, resistance to D-histidine can be used to score for mutants that are defective in general amino acid uptake. Resistance is scored on minimal medium containing 0·5 mg/ml proline as the sole nitrogen source and 0·5 mM-D-histidine.[158]

3-Aminotriazole

3-Aminotriazole is an inhibitor of the *HIS3* gene product, imidazoleglycerol phosphate dehydratase. Exposure of yeast cells to 3-AT causes histidine starvation, which in turn elicits the 'general control' response, resulting in transcriptional activation by Gcn4 of at least 35 genes encoding primarily amino acid biosynthetic enzymes.[73] 3-AT is commonly used to select or screen for mutants that are defective in the general control response. One class of mutants, designated *gcd*, are 3-AT-resistant due to constitutive derepression of *HIS3* and other general control-responsive genes. Conversely, *gcn* mutants are general control nonderepressible and are hypersensitive to 3-AT. 3-AT sensitivity is scored in SD medium containing 10–50 mM-3-AT.[123]

Sulfometuron methyl

Sulfometuron methyl (SM) is a herbicide that inhibits isoleucine and valine biosynthesis. As such, SM can be used to invoke the general control response by causing starvation for these two amino acids.[187] SM-resistant mutants have been described and include mutations in the *SMR1*, *SMR2* and *SMR3* genes.[51] *SMR1* is allelic to *ILV2*, which encodes an isoleucine/valine biosynthetic enzyme, and *smr2* is allelic to *PDR1*, a multidrug resistance gene.[51] SM-resistant mutants were selected on SD medium containing 3 μg/ml SM.[51]

Aminoglycoside antibiotics

Aminoglycoside antibiotics promote mistranslation of the genetic code in both prokaryotic and eukaryotic organisms, in many cases as a consequence of decreased translational fidelity during elongation. Several aminoglycosides, including hygromycin B, lividomycin, paromomycin and neomycin, promote phenotypic suppression of nonsense mutations in *S. cerevisiae*.[144,170] This effect does not involve genomic mutations; rather, phenotypic suppression is defined as a conditional loss of the mutant phenotype that is dependent upon the presence of the suppressing condition. These results suggest that altered sensitivities of yeast strains to aminoglycoside antibiotics can be useful screens for mutants defective in components of the translational apparatus.

There is broad variability in naturally occurring resistance to translational inhibitors.[1] It is therefore important to determine the minimal inhibitory concentration of aminoglycoside antibiotics before scoring mutants for increased resistance or sensitivity. This procedure and suggested drug concentrations for addition to YPD medium have been described previously.[1,49,123] Although mutations affecting translational fidelity are most often associated with increased *sensitivity* to aminoglycoside antibiotics, it is important to recognize that mutations conferring increased *resistance* have also been characterized. An alternative method for scoring sensitivity to aminoglycoside antibiotics is to dispense a solution of the drug to a sterile paper disk on solid media that has been seeded with the strain to be scored.[144,170] This allows for a strain to be scored for sensitivities to multiple antibiotics on a single plate.

Enhanced sensitivities of yeast mutants to a broad range of aminoglycoside antibiotics, including G-418, hygromycin B, destomycin A, gentamicin X2, apramycin, kanamycin B, lividomycin A, neamine, neomycin, paromomycin and tobramycin, were described by Ernst and Chan.[49] The genes defined in that study were designated *ags*, for aminoglycoside sensitive. In another study, *crl* mutants, which are cycloheximide resistant but lethal at 37°C, were screened for altered sensitivities to aminoglycoside antibiotics.[123] All *crl* mutants were found to be hypersensitive to hygromycin B and some exhibited sensitivity to cryptopleurine, anisomycin and G-418. Based on different aminoglycoside sensitivities, the *crl* mutations appear to be different from the *ags* mutations.[123] Most recently, sensitivity to paromomycin was found to be associated with the *mof4-1* allele of *UPF1*, which encodes a component of the nonsense-mediated mRNA decay pathway and is involved in reading frame maintenance.[40]

Although eukaryotic cells are naturally resistant to the aminoglycoside streptomycin, a yeast mutant displaying enhanced susceptibility to streptomycin has been described. A single base substitution within yeast 18S rRNA decreases resistance to streptomycin when rRNA is expressed solely from a plasmid-borne copy of the rDNA; interestingly, this base substitution occurs at the position equivalent to a substitution that confers streptomycin resistance in *Escherichia coli*.[33] This same yeast mutation also increases resistance to paromomycin and G-418.

Cycloheximide

Cycloheximide is a potent inhibitor of protein synthesis in eukaryotic cells and acts by binding to the 60S ribosomal subunit to inhibit both initiation and elongation. Mutants resistant to high concentrations of cycloheximide (>10 μg/ml) are the result of mutations in a single gene, *CYH2*, which encodes ribosomal protein L29. Growth inhibition also occurs in the presence of low levels of cycloheximide that do not completely inhibit protein synthesis. The effects on cell growth of low levels of cycloheximide (e.g. 1 μg/ml) are apparently due to an increase in the duration of the G_1 phase of the cell cycle.[124] Hunts for mutants resistant to low levels of cycloheximide have turned up strains that affect the cell cycle, protein synthesis, and permeability of the cell to cycloheximide.[124] In addition, a screen for mutants that are both resistant to low concentrations of cycloheximide and heat-lethal turned up mutations in genes designated *crl*. *crl* mutants have characteristics of both general control defects and omnipotent translational suppressors. These mutants were suggested to affect the fidelity of protein synthesis.[123] This establishes resistance to cycloheximide as an easily scored phenotype that at high concentrations is indicative of mutations in the *CYH2* gene, but at lower concentrations can be associated with a broad range of defects.

Trichodermin

Trichodermin is an antibiotic that inhibits peptidyltransferase activity. The isolation and characterization of trichodermin-resistant yeast mutants have been described.[55] Resistance was conferred by mutation in the *TCM1* gene, which encodes ribosomal protein L3. *tcm1* mutants are also resistant to structurally-distinct antibiotics that inhibit peptidyltransferase activity, including verrucarin A, anisomycin and sparsomycin. Furthermore, *tsm1* is allelic to *MAK8*, which was identified as a gene essential for the maintenance of the yeast killer phenotype.[190] Trichodermin medium consists of YPD plus 10 μg/ml trichodermin.[55] Anisomycin medium is YPD containing 20–50 μg/ml anisomycin.[123]

Immunosuppressants

Rapamycin, cyclosporin (CsA) and FK506 are immunosuppressive drugs that block signal transduction pathways involved in T-cell activation. They also exhibit antifungal properties.[102] Rapamycin, in particular, has been useful for selecting mutants that define components of signal transduction pathways in yeast. The effects of these drugs first requires their association with intracellular receptors. CsA binds cyclophilin and both rapamycin and FK506 bind the FK506 binding protein, FKBP. Cyclophilin and FKBP are proline isomerases. However, the effects of rapamycin, CsA and FK506 are not due to inhibition of the isomerase activity. Rather, CsA-cyclophilin and FK506-FKBP target calcineurin, a serine/threonine phosphatase, to interfere with Ca^{2+}-dependent signal transduction. In yeast, CsA-cyclophilin and FK506-FKBP block recovery from pheromone-induced G_1 arrest. The rapamycin–FKBP complex does not target calcineurin, but instead interacts directly with the phosphatidyl inositol (PI) 3-kinase family members Tor1 and Tor2 to block progression through G_1.[69,116] These two proteins were initially implicated as the targets of the rapamycin–FKBP complex based on the ability of *tor1* and *tor2* mutations to suppress the cytotoxic effect of rapamycin.[69]

Normal laboratory yeast strains are extremely sensitive to rapamycin, exhibiting growth arrest on YPD medium containing 0·1 μg/ml rapamycin.[69] Mutations in *FPR1*, which encodes the rapamycin receptor FKBP 12, suppress this effect, allowing cells to grow in the presence 100 μg/ml rapamycin.[69]

Neither CsA nor FK506 is as toxic as rapamycin and normal yeast strains exhibit varying degrees of susceptibility to both drugs. Nonetheless, yeast mutants exhibiting altered sensitivity to CsA and FK506 have been informative. For example, FK506 was found to inhibit amino acid import[68] and either overexpression of, or mutations in, the *TAP1/TAT1* and *TAP2/TAT2/SCM2* genes, which

encode amino acid permeases, affects FK506 sensitivity.[163]

Oligomycin

Oligomycin is an inhibitor of oxidative phosphorylation that blocks ADP-dependent stimulation of oxygen consumption. The *YOR1* gene encodes an ABC transporter that was identified as a high-copy-suppressor of oligomycin toxicity; conversely a *yor1* deletion confers hypersensitivity to oligomycin.[91] *YOR1* was also identified in a selection for reveromycin-sensitive mutants (see below).[41] Oligomycin resistance can be scored on YPGE medium containing 0·1 μg/ml oligomycin.[91]

o-*Dinitrobenzene*

o-Dinitrobenzene (*o*-DNB) is an agent that uncouples electron transport from oxidative phosphorylation. *o*-DNB is inactivated by covalent attachment to glutathione, catalysed by the enzyme glutathione-*S*-transferase. In an effort to identify genes involved in *o*-DNB detoxification in yeast, the *ROD1* gene was isolated as a high copy suppressor of *o*-DNB toxicity.[197] *ROD1* encodes a novel protein that conferred resistance not only to *o*-DNB, but also to high levels of calcium and zinc; conversely, a *rod1* deletion rendered cells sensitive to *o*-DNB, calcium, zinc and diamide. *o*-DNB resistance can be scored on YPD medium containing 175–400 μM-*o*-DNB.[197]

Multidrug resistance

Yeast, like mammalian cells, can acquire pleiotropic drug (multidrug) resistance. Two classes of genes are associated with this process. One encodes membrane transporter proteins such as the ATP binding cassette transporters (ABC transporters) that function as drug efflux pumps. The other class encodes transcription factors that activate expression of genes involve in drug detoxification. Examples include the *PDR5* gene (pleiotropic drug resistance), which encodes an ABC transporter, and the *PDR1* and *PDR3* genes, which encode zinc-finger transcription factors that control *PDR5* expression.[90] Multidrug resistance genes confer resistance to many distinct drugs, including actinomycin D, adriamycin, bleomycin, chloramphenicol, colchicine, cycloheximide, 5-fluorouracil and sulfometuron methyl, as well as resistance to the toxic effects of certain divalent cations. A single example, altered sensitivity to reveromycin, is described here.

Reveromycin is an anionic drug that inhibits progression through the G_1 phase of the mammalian cell cycle. Hypersensitive mutants have been isolated and characterized.[41] Mutations in the *YRS1/YOR1* gene, which encodes a homolog of the human multidrug resistance protein, were found to cause sensitivy to a broad range of organic anions, including the anionic drugs tautomycin and leptomycin B. However, *yrs1* mutants did not exhibit increased sensitivity to other drugs, including cycloheximide, fluphenazine, cerulenin and 4-nitroquinoline. The Ysr1 protein is structurally similar to Ycf1, which is required for resistance to cadmium, and *ysr1* mutants exhibit increased cadmium sensitivity. Sensitivity to reveromycin was scored on YPD medium, pH 4·5, containing 1 μg/ml reveromycin.[41] The low pH of the medium was critical, presumably because the cell membrane is more permeable to the protonated form of reveromycin.

CARBOHYDRATE AND LIPID BIOSYNTHESIS DEFECTS

Vanadate

Resistance to vanadate is a useful screen for mutants that are defective in protein glycosylation events.[8] Since glycosylation is tightly coupled to secretion, vanadate-resistant mutants have been informative with respect to both processes.[35] Although protein glycosylation occurs primarily in the Golgi, early glycosylation events occur in the endoplasmic reticulum. Interestingly, characterization of a vanadate-resistant, hygromycin B-sensitive mutant identified the *OST4* gene, which encodes a polypeptide of only 36 amino acids that is required for normal levels of oligosaccharyltransferase activity.[35] Vanadate resistance can be scored on YPD medium containing 7–10 μM-sodium ortho-vanadate.[35]

Fenpropimorph

Fenpropimorph is a fungicide that acts by inhibiting biosynthesis of ergosterol, the yeast counterpart of mammalian cholesterol.[104,120] Exposure of yeast cells to fenpropimorph results in accumulation of ergosterol precursors and inhibits cell growth by ergosterol starvation.[120] As is common for other forms of nutritional deprivation, fenpropimorph-induced ergosterol deprivation leads to a block in the G_1 phase of the cell cycle. Mutants resistant to fenpropimorph have been

isolated.[104] The *fen1-1* mutation enhances the level of ergosterol and causes a general resistance to sterol biosynthesis inhibitors. The *FEN1* gene and its homolog *SUR4* have been suggested to be involved in the dynamics of cortical actin cytoskeleton in response to nutrient availability.[151] Fenpropimorph resistance can be scored on SC medium. Whereas growth of a wild-type strain is inhibited by 0·3 μM-fenpropimorph, *fen1* mutants resist growth inhibition by 66 μM-fenpropimorph.[104,120]

Nystatin

Nystatin is a polyene antibiotic that binds to membrane ergosterol. Nystatin resistance is a hallmark of *erg* mutants, which are defective in ergosterol biosynthesis;[83] reviewed in reference 70. Resistance to nystatin can be scored on SD medium containing 1–6 units/ml nystatin.[83]

Resistance to other antifungal compounds, including amphotericin B (another polyene antibiotic) and syringomycin-E (a cyclic lipodepsipeptide),[179] is also associated with *erg* mutations. Therefore, resistance to these and other compounds is often indicative of defects in ergosterol biosynthesis.

Mevinolin and lovostatin

Mevolin and lovostatin are competitive inhibitors of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase. The isolation and initial characterization of mevinolin-resistance mutants of *S. cerevisive* have been reported.[10] All mevindin-resistant mutants were also slightly resistant to nystatin, a result consistent with the diminished sterol levels in these strains. Mevinolin-resistant mutants were isolated on YPD medium containing 400 μg/ml mevinolin, either in the presence or absence of exogenous ergosterol. Mutants were resistant to the same concentrations of mevinolin regardless of whether glucose or glycerol was the carbon source, demonstrating that resistance occurred under either fermentative or respiratory metabolism.[10]

NUCLEIC ACID METABOLISM DEFECTS

UV light

Sensitivity to UV irradiation is an easy phenotype to score for defects in repair of DNA damage. As examples, mutations in the *SSL1* and *SSL2* (*RAD25*) genes, which were initially identified based on suppression of a stem-loop structure in the leader region of the *HIS4* gene, confer sensitivity to UV irradiation.[63,199] The *SSL1* and *SSL2* genes were subsequently identified as subunits of the general transcription factor TFIIH, which also functions in nucleotide excision repair of DNA damage.[52,177] Sensitivity to UV irradiation is scored by plating parent and mutant strains on either SC or YPD medium and irradiating with either a calibrated dose or increasing doses of UV light. Typical doses for scoring sensitivity to UV irradiation are 10–200 Joules/m^2. A simple germicidal lamp or UV crosslinker is an adequate source of UV light for this purpose. Irradiated plates must be incubated in the dark for at least 24 h to eliminate activation of photo-induced repair.[67]

Alkylating agents

There are many examples of yeast strains that show hypersensitivity to different alkylating agents, including ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine, cisplatin and mitomycin C. As an example, *cdc2* mutations confer MMS sensitivity, presumably caused by failure of *cdc2*-encoded DNA polymerase δ to fill in single-strand gaps arising during base excision repair of methylation damage.[15] Sensitivity to EMS and MMS are particularly easy to score. For example, MMS sensitivity is scored on YPD medium containing 0·05% MMS.[122] Alternatively, chemical concentration gradients can be used in assays based on zonal growth inhibition, as described above for peroxide sensitivity.

Radiomimetic drugs

Bleomycin is a radiomimetic, antitumor drug that induces single- and double-strand DNA breaks through the production of free radicals. A screen for yeast mutants that are hypersensitive to bleomycin was recently described.[122] In that study, the *IMP2* gene was identified as the structural gene encoding a transcriptional activator that mediates protection against DNA damage caused by bleomycin as well as other oxidants.[122] Sensitivity of yeast strains to bleomycin can be scored on YPD medium containing 2–20 μg/ml bleomycin.

Yeast strains can also be scored for sensitivity to other radiomimetric compounds, including 4-nitroquinoline oxide (4-NQO) and streptonigrin. *rad52* mutants exhibit poor survival on YPD medium containing 0·5 μg/ml 4-NQO.[122]

Mutations in the functionally related *TEL1* and *MEC1* genes, which encode members of the PI 3-kinase family, confer sensitivity to streptonigrin, scored on YPD medium containing 0·5 μg/ml streptonigrin.[128] Neither *tel1* nor *mec1* alone conferred streptonigrin sensitivity, but a double *tel1 mec1* mutant was sensitive to both streptonigrin and bleomycin, as well as other DNA damaging agents.[128]

Hydroxyurea

Hydroxyurea (HU) is an inhibitor of ribonucleotide reductase, which catalyses the reduction of ribonucleotides to deoxyribonucleotides. Exposure of yeast cells to HU diminishes dNTP pools, thereby preventing DNA synthesis and progression through S phase of the cell cycle. There are many examples of HU-sensitive yeast mutants. One is the *crt* collection of mutants, which are constitutive for expression of *RNR3*, a highly regulated gene encoding a subunit of ribonucleotide reductase.[204] HU sensitivity can be scored on YPD medium containing 100 mM-HU.[204]

Distamycin A

Distamycin A binds to the minor groove of DNA with a preference for AT-rich sequences. Distamycin A and other minor groove ligands such as DAPI and Hoechst 33258 were found to be toxic to *S. cerevisiae*.[61] Consistent with the relatively AT-rich content of mitochondrial DNA, distamycin A was more toxic to yeast cells grown on glycerol medium, which requires a functional respiratory system, than to cells grown on glucose medium. Minimum inhibitory concentrations of distamycin A range from 80 to 400 μM with glucose as the carbon source and 4–20 μM with glycerol as the carbon source.[61]

Actinomycin D

Actinomycin D is a DNA intercalator with preference for GC-rich sequences. Exposure of yeast cells to actinomycin D has been reported to induce expression of *RNR3*, the highly inducible gene encoding a subunit of ribonucleotide reductase.[47] Exposure of yeast cells to 10 μM-actinomycin D in synthetic medium was sufficient to stimulate *RNR3* expression, although it was not reported whether this concentration of actinomycin D was sufficient to cause a growth phenotype.[47]

Camptothecin

Camptothecin is an anti-cancer drug that targets eukaryotic DNA topoisomerase I by reversibly trapping a covalent enzyme–DNA intermediate.[31] Consequently, camptohecin interferes with processes that involve topoisomerase I, including replication, transcription and recombination. The enzyme–DNA adduct interferes with replication, resulting in accumulation of double-stranded DNA breaks, which in turn lead to cell cycle arrest in G_2. Both camptothecin-sensitive and -resistant mutants of *S. cerevisiae* have been described. Mutations in the *TOP1* gene, which encodes topoisomerase I, confer camptothecin resistance, whereas overexpression of *TOP1* enhances sensitivity to camptothecin.[48,97,137] Consistent with the proposed model for camptothecin toxicity, *rad52* mutants, which are deficient in recombinational repair of double-stranded DNA breaks, exhibit increased sensitivity to camptothecin.[48,137] In a recent study, suppression of camptothecin-induced lethality identified dominant mutations in the *SCT1* gene.[92] Camptothecin medium is prepared by adding camptothecin dissolved in dimethylsulfoxide (or sodium camptothecin dissolved in water) to either YPD or minimal medium. Since camptothecin is less stable at acidic pH, medium should be buffered to pH 7·2–7·5.[137] Camptothecin-sensitive mutants were identified on YPD medium containing 5–50 μg/ml camptothecin;[137] suppressors of camptothecin sensitivity were selected on minimal medium containing 10 μg/ml camptothecin.[92]

Ciclopyroxolamine

Ciclopyroxolamine (CPX) is an inhibitor of mammalian DNA replication that causes arrest at the G_1/S stage of the cell cycle. CPX is also a broad-spectrum anti-fungal antibiotic, implying that it is permeable to yeast cells. Therefore, Levenson and Hamlin suggested that CPX sensitivity might be used to screen for yeast mutants that are altered in DNA replication. [107]

6-Azauracil

6-Azauracil (6-AU) is an inhibitor of both orotidylic acid decarboxylase and IMP dehydrogenase, which are components of the UTP and GTP biosynthetic pathways. Consequently, 6-AU diminishes the intracellular pools of UTP and GTP. A screen for mutants exhibiting sensitivity to 6-AU identified the *ppr1* and *ppr2* genes.[50] *PPR1*

encodes a transcriptional regulator of the pyrimidine pathway and the growth defect of a *ppr1* null mutant can be rescued by the addition of uracil to the growth medium.[50] *PPR2* encodes the transcription elongation factor TFIIS.[78,130] The sensitivity of *ppr2* mutants to 6-AU is thought to be a consequence of the TFIIS requirement of elongating RNA polymerase II under conditions of NTP deprivation.[50] Consistent with this interpretation, the sensitivity of *ppr2* mutants to 6-AU can be rescued by addition of uracil or guanine to the growth medium. Deletion of the *Sz. pombe* gene encoding TFIIS (*tfs1*) also confers sensitivity to 6-AU, which can be rescued by either uracil or guanine.[193] 6-AU-sensitive mutations were also uncovered in the *rbp1/rpo21* gene, which encodes the largest subunit of RNA polymerase II.[5] These mutants could be rescued either by increased dosage of the *PPR2* gene or by addition of guanine to the medium. These results suggested that functional interaction between RNA polymerase II and TFIIS is critical for elongation through pause sites. Therefore, sensitivity to 6-AU often correlates with defects in the elongation phase of transcription by RNA polymerase II. Sensitivity can be scored on minimal medium supplemented with 6-AU at concentrations of either 30 μg/ml in *S. cerevisiae*[5] or 300 μg/ml in *Sz. pombe*.[193]

Mycophenolic acid

Mycophenolic acid is an inhibitor of IMP dehydrogenase, an enzyme in the GTP biosynthetic pathway. Consequently, mycophenolic acid is assumed to diminish the intracellular pool of GTP. Consistent with this premise, mycophenolic acid sensitivity can be reversed by addition of guanine to the growth medium. Mutations in *PPR2* (TFIIS) result in increased sensitivity to mycophenolic acid, presumably due to the increased requirement by RNA polymerase II for TFIIS when the pool of NTP substrates is limiting.[147] Yeast strains are typically less sensitive to mycophenolic acid than to 6-AU (see above), perhaps because 6-AU diminishes the pools of both GTP and UTP.[50] Sensitivity to mycophenolic acid can be scored on YPD medium containing mycophenolic acid at a final concentration of 45 μg/ml.[147]

Thiolutin

Thiolutin is an inhibitor of RNA polymerase in yeast.[182] Thiolutin has been used in lieu of the ts *rbp1-1* allele as a means of shutting off *de novo* mRNA synthesis *in vivo*.[71] Conceivably, altered sensitivity to thiolutin might be a means to uncover RNA polymerase II mutants or other transcriptional defects. Thiolutin at a final concentration of 3 μg/ml was reported to inhibit RNA polymerase II transcription to <5% of normal.[71]

Inositol secretion (Opi)

As described above, inositol auxotrophy often correlates with defects in components of the general transcriptional apparatus, resulting in diminished expression of the *INO1* gene. Conversely, mutations in certain genes encoding transcriptional repressors cause overproduction and secretion of inositol.[62] This secretory phenotype, denoted Opi$^+$, correlates with overexpression of *INO1* and has been reported for deletions in the *OPI1, SIN3* and *UME6* genes, each of which encodes a transcriptional repressor.[79,81,189] The Opi$^+$ phenotype can be scored in a crossfeeding assay. For example, wild-type and mutant strains are allowed to grow on -Ino agar medium, followed by streaking a homozygous *ino1* diploid mutant away from these strains. Whereas the wild-type strain fails to rescue growth of the *ino1* mutant, repressor mutants secrete inositol, thereby crossfeeding the *ino1* mutants, scored as a streak of growth that diminishes with distance from the repressor mutant.[81]

Mutator phenotype

The potential for mutations to confer a mutator phenotype, defined by an enhanced rate of mutagenesis, can be conveniently assessed by scoring the frequency of resistance to certain toxins or antibiotics where resistance is known to arise by mutations in specific genes. For example, mutations in the *LYS2, CYH2, CAN1* and *URA3* genes confer resistance to α-aminoadipate, cycloheximide, canavanine, and 5-fluoro-orotic acid, respectively. Therefore a mutator phenotype can be scored by determining the frequency at which resistance to one or more of these compounds arises in a mutant strain relative to a wild-type control.[43] As an example of this phenotype, mutations in components of the replication machinery have been shown to confer increased frequency of resistance to α-aminoadipate.[21]

A FEW OTHER PHENOTYPES

pH-sensitivity

Mutants defective in vacuolar function and protein sorting often exhibit multiple pleiotropic phenotypes. Emr and colleagues reasoned that mutants defective in vacuole acidification might also be defective in regulation of intracellular pH.[9] Indeed, mutations in the *VPT13* gene, as well as mutations in other vacuole protein targeting genes, confer extreme sensitivity to low pH. *VPT13* mutants were also defective in accumulation of quinicrine in the vacuole. Sensitivity of *vpt* mutants to low pH were scored on YPD medium adjusted to pH 3·0 with 6-N-HCl.[9]

Sensitivity to benomyl, nocodazole and thiabendazole

Benomyl is an antimitotic drug that destablizes microtubules and has been shown to inhibit microtubule-mediated processes, including nuclear division, nuclear migration and nuclear fusion. As part of their efforts to understand microtubule function, Botstein and colleagues isolated and characterized yeast mutants based on either resistance or hypersensitivity to benomyl. All benomyl-resistant mutants were the result of mutations in a single gene, *TUB2*.[180] Benomyl-hypersensitive mutants fell into six complementation groups.[174] One group was composed of *TUB1, TUB2* and *TUB3*, the three tubulin structural genes. The other three genes were designated *CIN1, CIN2* and *CIN4*, genes that were also identified based on increased rates of chromosome loss. Additional experiments suggested that the three *CIN* genes act together in the same pathway or complex to affect microtubule function.[174] Other antimitotic drugs that destabilize microtubules include nocodazole and thiabendazole, both of which are toxic to yeast. Wild-type yeast strains grow well on YPD medium containing 10 μg/ml benomyl, whereas hypersensitive *tub* and *cin* mutants are inhibited on YPD medium containing as little as 0·5 μg/ml benomyl.[174] Benomyl sensitivity is temperature dependent, with increased sensitivity at lower temperatures.[174] Nocodazole sensitivity can be scored on YPD medium containing 0·25–4 μg/ml nocodazole;[174] sensitivity of *Sz. pombe* mutants to thiabendazole was scored on YPD medium containing 10–30 μg/ml thiabendazole.[168]

Staurosporine

Staurosporine is a protein kinase inhibitor that at low concentrations specifically inhibits protein kinase C.[136] Several different genes, designated *STT*, were identified in a hunt for mutants that are both staurosporine- and temperature-sensitive. Several of the *STT* genes have been characterized. *STT1* is identical to *PKC1*, the gene encoding protein kinase C, which activates signaling through the MAP kinase pathway.[200] Other *STT* genes are functionally related to *PKC1/STT1* and are involved in cell signaling and plasma membrane development.[201–203] Staurosporine sensitivity is scored on YPD medium containing 0·1 μg/ml staurosporine. Alternatively, staurosporine sensitivity can be conveniently scored in a halo assay by spotting 5 μl of 200 μg/ml staurosporine on a sterile filter disk in the center of YPD plates seeded with wild-type and mutant strains.[168]

Caffeine

Caffeine is a purine analog that affects many cellular processes. Growth sensitivity to caffeine is often associated with defects in components of MAP kinase pathways. As an example, mutations in *BRO1*, which encodes a protein that interacts with components of the Pkc1p–MAP kinase pathway, confers caffeine sensitivity.[134] Caffeine also inhibits mammalian cAMP phosphodiesterase, although it is not clear that caffeine has a similar inhibitory effect on *PDE1*- or *PDE2*- encoded cAMP phosphodiesterase in yeast. Caffeine sensitivity is typically scored on YPD medium containing 8–10 mM-caffeine.[54,134]

A FEW TRICKS

Cell permeabilization

Many drugs exert specific effects *in vitro* and would be potentially useful reagents in genetic selections or screens. However, these drugs are not toxic because they are impermeable to the cell. In some cases this problem can be alleviated by selecting for mutants that are permeable to other drugs. For example, permeability to camptothecin was increased by first selecting for a mutant with enhanced sensitivity to cycloheximide.[137] Other techniques can also be used to increase cell permeability. For example, Nitiss and Wang cite as unpublished results their use of yeast transformation protocols, including LiCl treatment, to enhance camptothecin permeability.[137]

Phenotypic enhancement

Mutant phenotypes are often 'leaky', which can make the phenotype difficult to follow through meiosis or render it useless as a selectable marker. However, the leaky phenotype of certain mutations can in some cases be enhanced by conditions that do not significantly affect the growth of the wild-type strain. One of the simplest methods to enhance a phenotype is to switch from rich (YPD) to synthetic complete (SC) medium.

Changes in growth temperature and osmotic pressure are additional methods that sometimes confer phenotypic enhancement. For example, the sensitivity of certain *crl* mutants to hygromycin B is enhanced by growth at elevated temperature (37°C) or by addition of 2·5 M-glycerol to the growth medium.[123] For some *crl* mutants, the combination of hygromycin B and either heat or glycerol abolished growth under conditions where neither condition alone conferred a phenotype.

Another method to enhance a phenotype is to supplement the growth medium with drugs or other reagents that have the potential to impair growth of a specific class of mutants. A recent example using this logic is provided by the *MCB1* gene, which encodes a multiubiquitin-chain-binding component of the 26S proteasome. Whereas disruption of *MCB1* had no growth defect on SC medium, addition of canavanine to the same medium conferred a severe growth defect, yet had minimal effect on growth of the isogenic wild-type strain.[185] Canavanine is an amino acid analog that is incorporated into proteins in the place of arginine, resulting in structural defects. It therefore stands to reason that deletion of a proteasome component involved in turnover of aberrant proteins would exhibit enhanced susceptibility to canavanine. This same logic can be applied to screen for other enhanced phenotypes.

ACKNOWLEDGEMENTS

I am especially grateful to David Gross for valuable advice on the content and organization of this review. I also thank Steve Brill, April Brys, Harry Duttweiler, Mike Leibowitz, Lenore Neigeborn, Zu-Wen Sun, Kelly Tatchell, Reed Wickner and Fred Winston for many helpful suggestions and comments on the manuscript. Research in my laboratory is supported by NIH grant GM-39484.

REFERENCES

1. Adoutte-Panvier, A. and Davies, J. E. (1984). Studies of ribosomes of yeast species: Susceptibility to inhibitors of protein synthesis *in vivo* and *in vitro*. *Mol. Gen. Genet.* **194,** 310–317.
2. Aguilera, A. (1994). Formamide sensitivity: a novel conditional phenotype in yeast. *Genetics* **136,** 87–91.
3. Aguilera, A. and Benitez, T. (1986). Ethanol-sensitive mutants of *Saccharomyces cerevisiae*. *Arch. Microbiol.* **143,** 337–344.
4. Apone, L. M., Virbasius, C. M. A., Reese, J. C. and Green, M. R. (1996). Yeast TAF(II)90 is required for cell-cycle progression through G(2)/M but not for general transcription activation. *Genes Devel.* **10,** 2368–2380.
5. Archambault, J., Lacroute, F., Ruet, A. and Friesen, J. D. (1992). Genetic interaction between transcription elongation factor TFIIS and RNA polymerase II. *Mol. Cell. Biol.* **12,** 4142–4152.
6. Arndt, K. M., Ricupero-Hovasse, S. and Winston, F. (1995). TBP mutants defective in activated transcription *in vivo*. *EMBO J.* **14,** 1490–1497.
7. Arndt, K. T., Styles, C. A. and Fink, G. R. (1989). A suppressor of a *HIS4* transcriptional defect encodes a protein with homology to the catalytic subunit of protein phosphatases. *Cell* **56,** 527–537.
8. Ballou, L., Hitzeman, R. A., Lewis, M. S. and Ballou, C. E. (1991). Vanadate-resistant yeast mutants are defective in protein glycosylation. *Proc. Natl. Acad. Sci. USA* **88,** 3209–3212.
9. Banta, L. M., Robinson, J. S., Klionsky, D. J. and Emr, S. D. (1988). Organelle assembly in yeast: characterization of yeast mutants defective in vacuolar biogenesis and protein sorting. *J. Cell. Biol.* **107,** 1369–1383.
10. Bard, M., Lees, N. D., Burnett, A. S. and Parker, R. A. (1988). Isolation and characterization of mevinolin resistant mutants of *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* **134,** 1071–1078.
11. Bartel, B. and Varshavsky, A. (1988). Hypersensitivity to heavy water: a new conditional phenotype. *Cell* **52,** 935–941.
12. Berroteran, R. W., Ware, D. E. and Hampsey, M. (1994). The *sua8* suppressors of *Saccharomyces cerevisiae* encode replacements of conserved residues within the largest subunit of RNA polymerase II and affect transcription start site selection similarly to *sua7* (TFIIB) mutations. *Mol. Cell. Biol.* **14,** 226–237.
13. Blacketer, M. J., Koehler, C. M., Coats, S. G., Myers, A. M. and Madaule, P. (1993). Regulation of dimorphism in *Saccharomyces cerevisiae*: involvement of the novel protein kinase homolog Elm1p and protein phosphatase 2A. *Mol. Cell. Biol.* **13,** 5567–5581.

14. Blacketer, M. J., Madaule, P. and Myers, A. M. (1995). Mutational analysis of morphologic differentiation in *Saccharomyces cerevisiae*. *Genetics* **140,** 1259–1275.
15. Blank, A., Kim, B. and Loeb, L. A. (1994). DNA polymerase delta is required for base excision repair of DNA methylation damage in *Saccharomyces cerevisiae*. *Proc, Natl. Acad. Sci. USA* **91,** 9047–9051.
16. Bonneu, M., Crouzet, M., Urdaci, M. and Aigle, M. (1991). Direct detection of yeast mutants with reduced viability on plates by erythrosine B staining. *Anal. Biochem*. **193,** 225–230.
17. Brandriss, M. C. (1979). Isolation and preliminary characterization of *Saccharomyces cerevisiae* proline auxotrophs. *J. Bacteriol.* **138,** 816–822.
18. Brandriss, M. C. and Magasanik, B. (1979). Genetics and physiology of proline utilization in *Saccharomyces cerevisiae*: mutation causing constitutive enzyme expression. *J. Bacteriol.* **140,** 504–507.
19. Brandriss, M. C. and Magasanik, B. (1981). Subcellular compartmentation in control of converging pathways for proline and arginine metabolism in *Saccharomyces cerevisiae*. *J. Bacteriol.* **145,** 1359–1364.
20. Brewster, J. L., de Valoir, T., Dwyer, N. D., Winter, E. and Gustin, M. C. (1993). An osmosensing signal transducing pathway in yeast. *Science* **259,** 1760–1763.
21. Brill, S., Maniar, H. S. and Wilson, R. (1997). Roles of replication protein-A subunits 2 and 3 in DNA replication fork movement in *Saccharomyces cerevisiae*. *Genetics* **145,** 891–902.
22. Brown, J. L. and Bussey, H. (1993). The yeast *KRE9* gene encodes an O-glycoprotein involved in cell surface beta-glucan assembly. *Mol. Cell. Biol.* **13,** 6346–6356.
23. Cameron, S., Levin, L., Zoller, M. and Wigler, M. (1988). cAmp-independent control of sporulation, glycogen metabolism, and heat shock resistance in *S. cerevisiae*. *Cell* **53,** 555–566.
24. Cannon, J. F., Pringle, J. R., Fiechter, A. and Khalil, M. (1994). Characterization of glycogen-deficient glc mutants of *Saccharomyces cerevisiae*. *Genetics* **136,** 485–503.
25. Carlson, M., Osmond, B. C. and Botstein, D. (1981). Mutants of yeast defective in sucrose utilization. *Genetics* **98,** 25–40.
26. Castro, C., Ribas, J. C., Valdivieso, M. H., Varona, R., del Rey, F. and Duran, A. (1995). Papulacandin B resistance in budding and fission yeasts: isolation and characterization of a gene involved in (1,3)beta-D-glucan synthesis in *Saccharomyces cerevisiae*. *J. Bacteriol.* **177,** 5732–5739.
27. Celenza, J. L., Marshall-Carlson, L. and Carlson, M. (1988). The yeast *SNF3* gene encodes a glucose transporter homologous to the mammalian protein. *Proc. Natl. Acad. Sci. USA* **85,** 2130–2134.
28. Chan, R. K. and Otte, C. A. (1982). Isolation and genetic analysis of *Saccharomyces cerevisiae* mutants supersensitive to G1 arrest by **a** factor and alpha factor pheromones. *Mol. Cell. Biol.* **2,** 11–20.
29. Chan, R. K. and Otte, C. A. (1982). Physiological characterization of *Saccharomyces cerevisiae* mutants supersensitive to G1 arrest by **a** factor and alpha factor pheromones. *Mol. Cell. Biol.* **2,** 21–29.
30. Chant, J. and Herskowitz, I. (1991). Genetic control of bud site selection in yeast by a set of gene products that constitute a morphogenetic pathway. *Cell* **65,** 1203–1212.
31. Chen, A. and Liu, L. (1994). *Annu. Rev. Pharmacol. Toxicol.* **84,** 191–218.
32. Chen, Y. and Piper, P. W. (1995). Consequences of the overexpression of ubiquitin in yeast: elevated tolerances of osmostress, ethanol and canavanine, yet reduced tolerances of cadmium, arsenite and paromomycin. *Biochim. Biophys. Acta.* **1268,** 59–64.
33. Chernoff, Y. O., Vincent, A. and Liebman, S. W. (1994). Mutations in eukaryotic 18S ribosomal RNA affect translational fidelity and resistance to aminoglycoside antibiotics. *EMBO J.* **13,** 906–913.
34. Chester, V. E. (1968). Heritable glycogen-storage deficiency in yeast and its induction by ultra-violet light. *J. Gen. Microbiol.* **51,** 49–56.
35. Chi, J. H., Roos, J. and Dean, N. (1996). The *OST4* gene of *Saccharomyces cerevisiae* encodes an unusually small protein required for normal levels of oligosaccharyltransferase activity. *J. Biol. Chem.* **271,** 3132–3140.
36. Choder, M. and Young, R. A. (1993). A portion of RNA polymerase II molecules has a component essential for stress responses and stress survival. *Mol. Cell. Biol.* **13,** 6984–6991.
37. Cooper, T. G. (1982). Transport in *Saccharomyces cerevisiae*. In Strathern, J. N., Jones, E. W. and Broach, J. R. (Eds), *The Molecular Biology of the Yeast Saccharomyces. Metabolism and Gene Expression.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 399 − 461.
38. Cooper, T. G. (1982). Nitrogen metabolism in *Saccharomyces cerevisiae*. In Strathern, J. N., Jones, E. W. and Broach, J. R. (Eds), *The Molecular Biology of the Yeast Saccharomyces. Metabolism and Gene Expression.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 39–99.
39. Cormack, B. P. and Struhl, K. (1993). Regional codon randomization: defining a TATA-binding protein surface required for RNA polymerase III transcription. *Science* **262,** 244–248.
40. Cui, Y., Dinman, J. D. and Peltz, S. W. (1996). *mof4-1* is an allele of the *UPF1/IFS2* gene which affects both mRNA turnover and -1 ribosomal frameshifting efficiency. *EMBO J.* **15,** 5726–5736.

41. Cui, Z., Hirata, D., Tsuchiya, E., Osada, H. and Miyakawa, T. (1996). The multidrug resistance-associated protein (MRP) subfamily (Yrs1/Yor1) of *Saccharomyces cerevisiae* is important for the tolerance to a broad range of organic anions. *J. Biol. Chem.* **271,** 14712–14716.
42. Culotti, J. and Hartwell, L. H. (1971). Genetic control of the cell division cycle in yeast. 3. Seven genes controlling nuclear division. *Exp. Cell Res.* **67,** 389–401.
43. Datta, A. and Jinks-Robertson, S. (1995). Association of increased spontaneous mutation rates with high levels of transcription in yeast. *Science* **268,** 1616–1619.
44. De Vendittis, E., Vitelli, A., Zahn, R. and Fasano, O. (1986). Suppression of defective RAS1 and RAS2 functions in yeast by an adenylate cyclase activated by a single amino acid change. *EMBO J.* **5,** 3657–3663.
45. Dickinson, J. R., Ambler, R. P. and Dawes, I. W. (1985). Abnormal amino acid metabolism in mutants of *Saccharomyces cerevisiae* affected in the initiation of sporulation. *Eur J. Biochem.* **148,** 405–406.
46. Dubois, E. and Grenson, M. (1979). Methylamine/ammonia uptake systems in *Saccharomyces cerevisiae*: multiplicity and regulation. *Mol. Gen. Genet.* **175,** 67–76.
47. Endo-Ichikawa, Y., Konho, H., Tokunaga, R. and Taketani, S. (1995). Induction in the gene *RNR3* in *Saccharomyces cerevisiae* upon exposure to different agents related to carcinogenesis. *Biochem Pharmacol.* **50,** 1695–1699.
48. Eng, W. K., Faucette, L., Johnson, R. K. and Sternglanz, R. (1988). Evidence that DNA topoisomerase I is necessary for the cytotoxic effects of camptothecin. *Mol. Pharmacol.* **34,** 755–760.
49. Ernst, J. F. and Chan, R. K. (1985). Characterization of *Saccharomyces cerevisiae* mutants supersensitive to aminoglycoside antibiotics. *J. Bacteriol.* **188,** 8–14.
50. Exinger, F. and Lacroute, F. (1992). 6-Azauracil inhibition of GTP biosynthesis in *Saccharomyces cerevisiae*. *Curr. Genet.* **22,** 9–11.
51. Falco, S. C. and Dumas, K. S. (1985). Genetic analysis of mutants of *Saccharomyces cerevisiae* resistant to the herbicide sulfometuron methyl. *Genetics* **109,** 21–35.
52. Feaver, W. J., Svejstrup, J. Q., Bardwell, L. *et al.* (1993). Dual roles of a multiprotein complex from *S. cerevisiae* in transcription and DNA repair. *Cell* **75,** 1379–1387.
53. Finley, D., Ciechanover, A. and Varshavsky, A. (1984). Thermolability of ubiquitin-activating enzyme from the mammalian cell cycle mutant ts85. **37,** 43–55.
54. Frederick, D. L. and Tatchell, K. (1996). The *REG2* gene of *Saccharomyces cerevisiae* encodes a type 1 protein phosphatase-binding protein that functions with Reg1p and the Snf1 protein kinase to regulate growth. *Mol. Cell. Biol.* **16,** 2922–2931.
55. Fried, H. M. and Warner, J. R. (1981). Cloning of yeast gene for trichodermin resistance and ribosomal protein L3. *Proc. Natl. Acad. Sci. USA* **78,** 238–242.
56. Gancedo, J. M. (1992). Carbon catabolite repression in yeast. *Eur. J. Biochem.* **206,** 297–313.
57. Gansheroff, L. J., Dollard, C., Tan, P. and Winston, F. (1995). The *Saccharomyces cerevisiae SPT7* gene encodes a very acidic protein important for transcription *in vivo*. *Genetics* **139,** 523–536.
58. Garrett, S. and Broach, J. (1989). Loss of Ras activity in *Saccharomyces cerevisiae* in suppressed by disruptions of a new kinase gene, *YAKI*, whose product may act downstream of the cAMP-dependent protein kinase. *Genes Devel.* **3,** 1336–1348.
59. Gimeno, C. J. and Fink, G. R. (1994). Induction of pseudohyphal growth by overexpression of *PHD1*, a *Saccharomyces cerevisiae* gene related to transcriptional regulators of fungal development. *Mol. Cell. Biol.* **14,** 2100–2112.
60. Gimeno, C. J., Ljungdahl, P. O., Styles, C. A. and Fink, G. R. (1992). Unipolar cell divisions in the yeast *S. cerevisiae* lead to filamentous growth: regulation by starvation and RAS. *Cell* **68,** 1077–1090.
61. Ginsburg, H., Nissani, E., Krugliak, M. and Williamson, D. H. (1993). Selective toxicity to malaria parasites by non-intercalating DNA-binding ligands. *Mol. Biochem. Parasitol.* **58,** 7–15.
62. Greenberg, M. L., Goldwasser, P. and Henry, S. A. (1982). Characterization of a yeast regulatory mutant constitutive for synthesis of inositol-1-phosphate synthase. *Mol. Gen. Genet.* **186,** 157–163.
63. Gulyas, K. D. and Donahue, T. F. (1992). *SSL2*, a suppressor of a stem-loop mutation in the *HIS4* leader encodes the yeast homolog of human ERCC-3. *Cell* **69,** 1031–1042.
64. Guthrie, C., Nashimoto, H. and Nomura, M. (1969). Structure and function of *E. coli* ribosomes, VIII. Cold-sensitive mutants defective in ribosome assembly. *Proc. Natl. Acad. Sci. USA*. **63,** 384–391.
65. Han, M., Kim, U. J., Kayne, P. and Grunstein, M. (1988). Depletion of histone H4 and nucleosomes activates the *PHO5* gene in *Saccharomyces cerevisiae*. *EMBO J.* **7,** 2221–2228.
66. Hartwell, L. H. (1967). Macromolecule synthesis in temperature-sensitive mutants of yeast. *J. Bacteriol.* **93,** 1662–1670.
67. Haynes, R. H. and Kunz, B. A. (1981). DNA repair and mutagenesis in yeast. In Strathern, J. N., Jones, E. W. and Broach, J. R. (Eds), *The Molecular Biology of the Yeast Saccharomyces.*

Metabolism and Gene Expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 371–414.
68. Heitman, J., Koller, A., Kunz, J. *et al.* (1993). The immunosuppressant FK506 inhibits amino acid import in *Saccharomyces cerevisiae. Mol. Cell. Biol.* **13,** 5010–5019.
69. Heitman, J., Movva, N. R. and Hall, M. N. (1991). Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* **253,** 905–909.
70. Henry, S. A. (1982). Membrane lipids of yeast: biological and genetic studies. In Strathern, J. N., Jones, E. W. and Broach, J. R. (Eds), *The Molecular and Cellular Biology of the Yeast Saccharomyces. Metabolism and Gene Expression.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 101–158.
71. Herrick, D., Parker, R. and Jacobson, A. (1990). Identification and comparison of stable and unstable mRNAs in *Saccharomyces cerevisiae. Mol. Cell. Biol.* **10,** 2269–2284.
72. Herskowitz, I. (1988). Life cycle of the budding yeast *Saccharomyces cerevisiae. Microbiol. Rev.* **52,** 536–553.
73. Hinnebusch, A. (1992). General and pathway-specific regulatory mechanisms controlling the synthesis of amino acid biosynthetic enzymes in *Saccharomyces cerevisiae*. In Jones, E., Pringle, J. and Broach, J. (Eds), *The Molecular and Cellular Biology of the Yeast Saccharomyces*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 319–414.
74. Hinnebusch, A. G. (1988). Mechanisms of gene regulation in the general control of amino acid biosynthesis in *Saccharomyces cerevisiae. Microbiol. Rev.* **52,** 248–273.
75. Hong, Z., Mann, P., Brown, N. H. *et al.* (1994). Cloning and characterization of *KNR4*, a yeast gene involved in (1,3)-beta-glucan synthesis. *Mol. Cell. Biol.* **14,** 1017–1025.
76. Horowitz, N. H. and Leupold, U. (1951). Some recent studies bearing on the one gene–one enzyme hypothesis. *Cold Spring Harbor Symp. Quant. Biol.* **16,** 65–72.
77. Huang, D., Chun, K. T., Goebl, M. G. and Roach, P. J. (1996). Genetic interactions between *REG1*/*HEX2* and *GLC7*, the gene encoding the protein phosphatase type 1 catalytic subunit in *Saccharomyces cerevisiae. Genetics* **143,** 119–127.
78. Hubert, J. C., Guyonvarch, A. Kammerer, B., Exinger, F., Liljelund, P. and Lacroute, F. (1983). Complete sequence of a eukaryotic regulatory gene. *EMBO J.* **2,** 2071–2073.
79. Hudak, K. A., Lopes, J. M. and Henry, S. A. (1994). A pleiotropic phospholipid biosynthetic regulatory mutation in *Saccharomyces cerevisiae* is allelic to *sin3* (*sdi1*, *ume4*, *rpd1*). *Genetics* **136,** 475–483.
80. Hutchins, K. and Bussey, H. (1983). Cell wall receptor for yeast killer toxin: involvement of (1→6)-beta-D-glucan. *J. Bacteriol.* **154,** 161–169.
81. Jackson, J. C. and Lopes, J. M. (1996). The yeast *UME6* gene is required for both negative and positive transcriptional regulation of phospholipid biosynthetic gene expression. *Nucl. Acids Res.* **24,** 1322–1329.
82. Jarvik, J. and Botstein, D. (1975). Conditional-lethal mutations that suppress genetic defects in morphogenesis by altering structural properties. *Proc. Natl. Acad. Sci. USA* **72,** 2738–2742.
83. Jiang, B., Brown, J. L., Sheraton, J., Fortin, N. and Bussey, H. (1994). A new family of yeast genes implicated in ergosterol synthesis is related to the human oxysterol binding protein. *Yeast* **10,** 341–353.
84. Jiang, B., Ram, A. F., Sheraton, J., Klis, F. M. and Bussey, H. (1995). Regulation of cell wall beta-glucan assembly: *PTC1* negatively affects *PBS2* action in a pathway that includes modulation of *EXG1* transcription. *Mol. Gen. Genet.* **248,** 260–269.
85. Johnston, M. (1996). Genome sequencing: The complete code for a eukaryotic cell. *Curr. Bol.* **6,** 500–503.
86. Johnston, M. and Carlson, M. (1992). Regulation of carbon and phosphate utilization. In Jones, E., Pringle, J. and Broach, J. (Eds), *The Molecular and Cellular Biology of the Yeast Saccharomyces*. Cold Spring Harbor Press, Cold Spring Harbor, NY, pp. 193–281.
87. Johnston, M. and Davis, R. W. (1984). Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae. Mol. Cell. Biol.* **4,** 1440–1448.
88. Kaiser, C., Michaelis, S. and Mitchell, A. (1994). *Methods in Yeast Genetics.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
89. Karpova, T. S., Lepetit, M. M. and Cooper, J. A. (1993). Mutations that enhance the *cap2* null mutant phenotype in *Saccharomyces cerevisiae* affect the actin cytoskeleton, morphogenesis and pattern of growth. *Genetics* **135,** 693–709.
90. Katzmann, D. J., Burnett, P. E., Golin, J., Mahe, Y. and Moye-Rowley, W. S. (1994). Transcriptional control of the yeast *PDR5* gene by the *PDR3* gene product. *Mol. Cell. Biol.* **14,** 4653–4661.
91. Katzmann, D. J., Hallstrom, T. C., Voet, M. *et al.* (1995). Expression of an ATP-binding cassette transporter-encoding gene (*YOR1*) is required for oligomycin resistance in *Saccharomyces cerevisiae. Mol. Cell. Biol.* **15,** 6875–6883.
92. Kauh, E. A., and Bjornsti, M. A. (1995). *SCT1* mutants suppress the camptothecin sensitivity of yeast cells expressing wild-type DNA topoisomerase I. *Proc. Natl. Acad. Sci. USA* **92,** 6299–6303.

93. Keleher, C. A., Redd, M. J., Schultz, J., Carlson, M. and Johnson, A. D. (1992). Ssn6-Tup1 is a general repressor of transcription in yeast. *Cell* **68,** 709–719.
94. Kim, H. B., Haarer, B. K. and Pringle, J. R. (1991). Cellular morphogenesis in the *Saccharomyces cerevisiae* cell cycle: localization of the *CDC3* gene product and the timing of events at the budding site. *J. Cell. Biol.* **112,** 535–544.
95. Kim, J. and Michels, C. A. (1988). The *MAL63* gene of *Saccharomyces* encodes a cysteine-zinc finger protein. *Curr. Genet.* **14,** 319–323.
96. Kim, K. S., Rosenkrantz, M. S. and Guarente, L. (1986). *Saccharomyces cerevisiae* contains two functional citrate synthase genes. *Mol. Cell. Biol.* **6,** 1936–1942.
97. Knab, A. M., Fertala, J. and Bjornsti, M. A. (1993). Mechanisms of camptothecin resistance in yeast DNA topoisomerase I mutants. *J. Biol. Chem.* **268,** 22322–22330.
98. Knaus, R., Pollock, R. and Guarente, L. (1996). Yeast *SUB1* is a suppressor of TFIIB mutations and has homology to the human co-activator PC4. *EMBO J.* **15,** 1933–1940.
99. Krems, B., Charizanis, C. and Entian, K. D. (1995). Mutants of *Saccharomyces cerevisiae* sensitive to oxidative and osmotic stress. *Curr. Genet.* **27,** 427–434.
100. Krems, B., Charizanis, C. and Entian, K. D. (1996). The response regulator-like protein Pos9/Skn7 of *Saccharomyces cerevisiae* is involved in oxidative stress resistance. *Curr. Genet.* **29,** 327–334.
101. Krisak, L., Strich, R., Winters, R. S. *et al.* (1994). *SMK1*, a developmentally regulated MAP kinase, is required for spore wall assembly in *Saccharomyces cerevisiae*. *Genes Devel.* **8,** 2151–2161.
102. Kunz, J. and Hall, M. N. (1993). Cyclosporin A, FK506 and rapamycin: more than just immunosuppression. *Trends Biochem. Sci.* **18,** 334–338.
103. Kushnir, S., Babiychuk, E., Kampfenkel, K., Belles-Boix, E., Van Montagu, M. and Inze, D. (1995). Characterization of *Arabidopsis thaliana* cDNAs that render yeasts tolerant toward the thiol-oxidizing drug diamide. *Proc. Natl. Acad. Sci. USA* **92,** 10580–10584.
104. Ladeveze, V., Marcireau, C., Delourme, D. and Karst, F. (1993). General resistance to sterol biosynthesis inhibitors in *Saccharomyces cerevisiae*. *Lipids* **28,** 907–912.
105. Lauder, S., Bankmann, M., Guzder, S. N., Sung, P., Prakash, L. and Prakash, S. (1996). Dual requirement for the yeast *MMS19* gene in DNA repair and RNA polymerase II transcription. *Mol. Cell. Biol.* **16,** 6783–6793.
106. Lenburg, M. E. and O'Shea, E. K. (1996). Signaling phosphate starvation. *Trends Biochem. Sci.* **21,** 383–387.
107. Levenson, V. and Hamlin, J. L. (1993). A general protocol for evaluating the specific effects of DNA replication inhibitors. *Nucl. Acids Res.* **21,** 3997–4004.
108. Levin, N. A., Bjornsti, M. A. and Fink, G. R. (1993). A novel mutation in DNA topoisomerase I of yeast causes DNA damage and *RAD9*-dependent cell cycle arrest. *Genetics* **133,** 799–814.
109. Lew, D. and Reed, S. (1995). Cell cycle control of morphogenesis in budding yeast. *Curr. Opin. Genet. Dev.* **5,** 17–23.
110. Liao, X. and Butow, R. A. (1993). *RTG1* and *RTG2*: two yeast genes required for a novel path of communication from mitochondria to the nucleus. *Cell* **72,** 61–71.
111. Lillie, S. H. and Pringle, J. R. (1980). Reserve carbohydrate metabolism in *Saccharomyces cerevisiae*: responses to nutrient limitation. *J. Bacteriol.* **143,** 1384–1394.
112. Lin, S. J. and Culotta, V. C. (1995). The *ATX1* gene of *Saccharomyces cerevisiae* encodes a small metal homeostasis factor that protects cells against reactive oxygen toxicity. *Proc. Natl. Acad. Sci. USA* **92,** 3784–3788.
113. Liu, H., Styles, C. A. and Fink, G. R. (1993). Elements of the yeast pheromone response pathway required for filamentous growth of diploids. *Science* **262,** 1741–1744.
114. Liu, X. D. and Thiele, D. J. (1996). Oxidative stress induced heat shock factor phosphorylation and HSF-dependent activation of yeast metallothionein gene transcription. *Genes Devel.* **10,** 592–603.
115. Ljungdahl, P. O., Gimeno, C. J., Styles, C. A. and Fink, G. R. (1992). *SHR3*: a novel component of the secretory pathway specifically required for localization of amino acid permeases in yeast. *Cell* **71,** 463–478.
116. Lorenz, M. C. and Heitman, J. (1995). TOR mutations confer rapamycin resistance by preventing interaction with FKBP12-rapamycin. *J. Biol. Chem.* **270,** 27531–27537.
117. Lovett, S. T. and Mortimer, R. K. (1987). Characterization of null mutants of the *RAD55* gene of *Saccharomyces cerevisiae*: effects of temperature, osmotic strength and mating type. *Genetics* **116,** 547–553.
118. Lussier, M., Sdicu, A. M., Camirand, A. and Bussey, H. (1996). Functional characterization of the *YUR1*, *KTR1*, and *KTR2* genes as members of the yeast *KRE2*/*MNT1* mannosyltransferase gene family. *J. Biol. Chem.* **271,** 11001–11008.
119. Magasanik, B. (1992). Regulation of nitrogen utilization. In Jones, E. W., Pringle, J. R. and Broach, J. R. (Eds), *The Molecular and Cellular Biology of the Yeast Saccharomyces*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 283–317.

120. Marcireau, C., Guilloton, M. and Karst, F. (1990). *In vivo* effects of fenpropimorph on the yeast *Saccharomyces cerevisiae* and determination of the molecular basis of the antifungal property. *Antimicrob. Agents Chemother.* **34,** 989–993.
121. Marczak, J. E. and Brandriss, M. C. (1989). Isolation of constitutive mutations affecting the proline utilization pathway in *Saccharomyces cerevisiae* and molecular analysis of the *PUT3* transcriptional activator. *Mol. Cell. Biol.* **9,** 4696–4705.
122. Masson, J.-Y. and Ramotar, D. (1996). The *Saccharomyces IMP2* gene encodes a transcriptional activator that mediates protection against DNA damage caused by bleomycin and other oxidants. *Mol. Cell. Biol.* **16,** 2091–2100.
123. McCusker, J. H. and Haber, J. E. (1988). *crl* mutants of *Saccharomyces cerevisiae* resemble both mutants affecting general control of amino acid biosynthesis and omnipotent translational suppressor mutants. *Genetics* **119,** 317–327.
124. McCusker, J. H. and Haber, J. E. (1988). Cycloheximide-resistant temperature-sensitive lethal mutations of *Saccharomyces cerevisiae*. *Genetics* **119,** 303–315.
125. McKenzie, E. A., Kent, N. A., Dowell, S. J., Moreno, F., Bird, L. E. and Mellor, J. (1993). The centromere and promoter factor-1, *CPF1*, of *Saccharomyces cerevisiae* modulates gene activity through a family of factors including *SPT21*, *RPD1* (*SIN3*), *RPD3* and *CCR4*. *Mol. Gen. Genet.* **240,** 374–386.
126. Miller, M. E., Cairns, B. E., Levinson, R. S., Yamamoto, K. R., Engel, D. A. and Smith, M. M. (1996). Adenovirus E1A specifically blocks SWI/SNF-dependent transcriptional activation. *Mol. Cell. Biol.* **16,** 5737–5743.
127. Mitsuzawa, H., Uno, I., Oshima, T. and Ishikawa, T. (1989). Isolation and characterization of temperature-sensitive mutations in the *RAS2* and *CYR1* genes of *Saccharomyces cerevisiae*. *Genetics* **123,** 739–748.
128. Morrow, D. M., Tagle, D. A., Shiloh, Y., Collins, F. S. and Hieter, P. (1995). *TEL1*, an *S. cerevisiae* homolog of the human gene mutated in ataxia telangiectasia, is functionally related to the yeast checkpoint gene *MEC1*. *Cell* **82,** 831–840.
129. Na, J. G., Pinto, I. and Hampsey, M. (1992). Isolation and characterization of *SUA5*, a novel gene required for normal growth in *Saccharomyces cerevisiae*. *Genetics* **131,** 791–801.
130. Nakanishi, T., Nakano, A., Nomura, K., Sekimizu, K. and Natori, S. (1992). Purification, gene cloning, and gene disruption of the transcription elongation factor S-II in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **267,** 13200–13204.
131. Nasmyth, K. (1993). Control of the yeast cell cycle by the Cdc28 protein kinase. *Curr. Opin. Cell Biol.* **5,** 166–179.
132. Neigeborn, L. and Carlson, M. (1984). Genes affecting the regulation of *SUC2* gene expression by glucose repression in *Saccharomyces cerevisiae*. *Genetics* **108,** 845–858.
133. Neigeborn, L. and Carlson, M. (1987). Mutations causing constitutive invertase synthesis in yeast: genetic interactions with *snf* mutations. *Genetics* **115,** 247–253.
134. Nickas, M. E. and Yaffe, M. P. (1996). *BRO1*, a novel gene that interacts with components of the Pkc1p-mitogen-activated protein kinase pathway in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **16,** 2585–2593.
135. Nikoloff, D. M. and Henry, S. A. (1991). Genetic analysis of yeast phospholipid biosynthesis. *Annu. Rev. Genet*. **25,** 559–583.
136. Nishizuka, Y. (1986). Studies and perspectives of protein kinase C. *Science* **233,** 305–312.
137. Nitiss, J. and Wang, J. C. (1988). DNA topoisomerase-targeting antitumor drugs can be studied in yeast. *Proc. Natl. Acad. Sci. USA* **85,** 7501–7505.
138. Novick, P. and Botstein, D. (1985). Phenotypic analysis of temperature-sensitive yeast actin mutants. *Cell* **40,** 405–416.
139. Ohya, Y., Miyamoto, S., Ohsumi, Y. and Anraku, Y. (1986). Calcium-sensitive *cls4* mutant of *Saccharomyces cerevisiae* with a defect in bud formation. *J. Bacteriol.* **165,** 28–33.
140. Ohya, Y., Ohsumi, Y. and Anraku, Y. (1984). Genetic study of the role of calcium ions in the cell division cycle of *Saccharomyces cerevisiae*: a calcium-dependent mutant and its trifluoperazine-dependent pseudorevertants. *Mol. Gen. Genet*. **193,** 389–394.
141. Ohya, Y., Ohsumi, Y. and Anraku, Y. (1986). Isolation and characterization of Ca^{2+}-sensitive mutants of *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* **132,** 979–988.
142. Oshima, Y. (1982). Regulatory circuits for gene expression: the metabolism of galactose and phosphate. In Strathern, J., Jones, E. and Broach, J. (Eds), *The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 159–180.
143. Ozcan, S., Vallier, L. G., Flick, J. S., Carlson, M. and Johnston, M. (1997). Expression of the *SUC2* gene of *Saccharomyces cerevisiae* is induced by low levels of glucose. *Yeast* **13,** 127–137.
144. Palmer, E., Wilhelm, J. M. and Sherman, F. (1979). Phenotypic suppression of nonsense mutants in yeast by aminoglycoside antibiotics *Nature* **277,** 148–150.
145. Pinto, I., Ware, D. E. and Hampsey, M. (1992). The yeast *SUA7* gene encodes a homolog of human transcription factor TFIIB and is required for normal start site selection *in vivo*. *Cell* **68,** 977–988.

146. Piper, P. W. (1995). The heat shock and ethanol stress responses of yeast exhibit extensive similarity and functional overlap. *FEMS Microbiol. Lett.* **134,** 121–127.
147. Powell, W., Bartholomew, B. and Reines, D. (1996). Elongation factor SII contacts the 3′-end of RNA in the RNA polymerase II elongation complex. *J. Biol. Chem.* **271,** 22301–22304.
148. Pringle, J. R. (1991). Staining of bud scars and other cell wall chitin with calcofluor. *Methods Enzymol.* **194,** 732–735.
149. Pringle, J. R. and Hartwell, L. H. (1981). The *Saccharomyces cerevisiae* life cycle. In Strathern, J. N., Jones, E. W. and Broach, J. R. (Eds), *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY, pp. 97–142.
150. Reed, S. I. (1992). The role of p34 kinases in the G1 to S-phase transition. *Annu. Rev. Cell Biol.* **8,** 529–561.
151. Revardel, E., Bonneau, M., Durrens, P. and Aigle, M. (1995). Characterization of a new gene family developing pleiotropic phenotypes upon mutation in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta.* **1263,** 261–265.
152. Roberts, R. L. and Fink, G. R. (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 Devel.* **8,** 2974–2985.
153. Roberts, S. M. and Winston, F. (1996). *SPT20*/*ADA5* encodes a novel protein functionally related to the TATA-binding protein and important for transcription in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **16,** 3206–3213.
154. Roncero, C., Valdivieso, M. H., Ribas, J. C. and Duran, A. (1988). Isolation and characterization of *Saccharomyces cerevisiae* mutants resistant to Calcofluor white. *J. Bacteriol.* **170,** 1950–1954.
155. Roon, R. J., Even, H. L., Dunlop, P. and Larimore, F. L. (1975). Methylamine and ammonia transport in *Saccharomyces cerevisiae*. *J. Bacteriol.* **122,** 502–509.
156. Roon, R. J., Levy, J. S. and Larimore, F. (1977). Negative interactions between amino acid and methylamine/ammonia transport systems of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **252,** 3599–3604.
157. Rowen, D. W., Meinke, M. and LaPorte, D. C. (1992). *GLC3* and *GHA1* of *Saccharomyces cerevisiae* are allelic and encode the glycogen branching enzyme. *Mol. Cell. Biol.* **12,** 22–29.
158. Rytka, J. (1975). Positive selection of general amino acid permease mutants in *Saccharomyces cerevisiae*. *J. Bacteriol.* **121,** 562–570.
159. Sanders, S. L. and Field, C. M. (1995). Cell Division. Bud-site selection is only skin deep. *Curr. Biol.* **5,** 1213–1215.
160. Sass, P., Field, J., Nikawa, J., Toda, T. and Wigler, M. (1986). Cloning and characterization of the high affinity cAMP phophodiesterase of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **83,** 9303–9307.
161. Scheraga, H. A., Nemethy, G. and Steinberg, I. Z. (1962). The contribution of hydrophobic bonds to the thermal stability of protein conformations. *J. Biol. Chem.* **237,** 2506–2508.
162. Schmid, A., Fascher, K. D. and Horz, W. (1992). Nucleosome disruption at the yeast *PHO5* promoter upon *PHO5* induction occurs in the absence of DNA replication. *Cell* **71,** 853–864.
163. Schmidt, A., Hall, M. N. and Koller, A. (1994). Two FK506 resistance-conferring genes in *Saccharomyces cerevisiae*, *TAT1* and *TAT2*, encode amino acid permeases mediating tyrosine and tryptophan uptake. *Mol. Cell. Biol.* **14,** 6597–6606.
164. Schultz, J. and Carlson, M. (1987). Molecular analysis of *SSN6*, a gene functionally related to the *SNF1* protein kinase of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **7,** 3637–3645.
165. Schwob, E., Bohm, T., Mendenhall, M. D. and Nasmyth, K. (1994). the B-type cyclin kinase inhibitor p40SIC1 controls the G1 to S transition in *S. cerevisiae*. *Cell* **79,** 233–244.
166. Sherman, F. (1991). Getting started with yeast. *Methods. Enzymol.* **194,** 3–21.
167. Shilo, V., Simchen, G. and Shilo, B. (1978). Initiation of meiosis in cell cycle initiation mutants of *Saccharomyces cerevisiae*. *Exp. Cell. Res.* **112,** 241–248.
168. Shimanuki, M., Goebl, M., Yanagida, M. and Toda, T. (1992). Fission yeast sts1+ gene encodes a protein similar to the chicken lamin B receptor and is implicated in pleiotropic drug-sensitivity, divalent cation-sensitivity, and osmoregulation. *Mol. Biol. Cell.* **3,** 263–273.
169. Simchen, G. and Kassir, Y. (1989). Genetic regulation of differentiation towards meiosis in the yeast *Saccharomyces cerevisiae*. *Genome* **31,** 95–99.
170. Singh, A., Ursic, D. and Davies, J. (1979). Phenotypic suppression and misreading *Saccharomyces cerevisiae*. *Nature* **277,** 146–148.
171. Snyder, M., Gehrung, S. and Page, B. D. (1991). Studies concerning the temporal and genetic control of cell polarity in *Saccharomyces cerevisiae*. *J. Cell Biol.* **114,** 515–532.
172. Song, W., Treich, I. Qian, N. Kuchin, S. and Carlson, M. (1996). *SSN* genes that affect transcriptional repression in *Saccharomyces cerevisiae* encode *SIN4*, *ROX3*, and *SRB* proteins associated with RNA polymerase II. *Mol. Cell. Biol.* **16,** 115–120.
173. Sprague Jr., G. F. and Herskowitz, I. (1981). Control of yeast cell type by the mating type locus. I. Identification and control of expression of the **a**-specific gene, *BAR1*. *J. Mol. Biol.* **153,** 305–321.

174. Stearns, T., Hoyt, M. A. and Botstein, D. (1990). Yeast mutants sensitive to antimicrotubule drugs define three genes that affect microtubule function. *Genetics* **124,** 251–262.
175. Sun, Z. W. and Hampsey, M. (1995). Identification of the gene (*SSU71*/*TFG1*) encoding the largest subunit of transcription factor TFIIF as a suppressor of a TFIIB mutation in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **92,** 3127–3131.
176. Svaren, J. and Horz, W. (1997). Transcription factors vs nucleosomes: regulation of the *PHO5* promoter of yeast. *Trends Biochem. Sci.* **22,** 93–97.
177. Svejstrup, J. Q., Wang, Z. G., Feaver, W. J. *et al.* (1995). Different forms of TFIIH for transcription and DNA repair: Holo-TFIIH and a nucleotide excision repairosome. *Cell* **80,** 21–28.
178. Tai, P.-C., Kessler, D. P. and Ingraham, J. (1969). Cold-sensitive mutations in *Salmonella typhimurium* which affect ribosome synthesis. *J. Bacteriol.* **97,** 1298–1304.
179. Takemoto, J. Y., Yu, Y., Stock, S. D. and Miyakawa, T. (1993). Yeast genes involved in growth inhibition by *Pseudomonas syringae pv. syringae* syringomycin family lipodepsipeptides. *FEMS Microbiol. Lett.* **114,** 339–342.
180. Thomas, J. H., Neff, N. F. and Botstein, D. (1985). Isolation and characterization of mutations in the beta-tubulin gene of *Saccharomyces cerevisiae*. *Genetics* **111,** 715–734.
181. Thompson, C. M. and Young, R. A. (1995). General requirement for RNA polymerase II holoenzymes *in vivo*. *Proc. Natl. Acad. Sci. USA* **92,** 4587–4590.
182. Tipper, D. J. (1973). Inhibition of yeast ribonucleic acid polymerases by thiolutin. *J. Bacteriol.* **116,** 245–256.
183. Toda, T., Uno, I., Ishikawa, T. *et al.* (1985). In yeast, RAS proteins are controlling elements of adenylate cyclase. *Cell* **40,** 27–36.
184. Tzagoloff, A. and Dieckmann, C. L. (1990). *PET* genes of *Saccharomyces cerevisiae*. *Microbiol. Rev.* **54,** 211–225.
185. van Nocker, S., Sadis, S., Rubin, D. M. *et al.* (1996). The multiubiquitin-chain-binding protein Mcb1 is a component of the 26S proteosome in *Saccharomyces cerevisiae* and plays a nonessential, substrate-specific role in protein turnover. *Mol. Cell. Biol.* **16,** 6020–6028.
186. van Zyl, W., Huang, W., Sneddon, A. A. *et al.* (1992). Inactivation of the protein phosphatase 2A regulatory subunit A results in morphological and transcriptional defects in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12,** 4946–4959.
187. Vazquez de Aldana, C. R., Wek, R. C., Segundo, P. S., Truesdell, A. G. and Hinnebusch, A. G. (1994). Multicopy tRNA genes functionally suppress mutations in yeast eIF-2 alpha kinase GCN2: evidence for separate pathways coupling GCN4 expression to unchanges tRNA. *Mol. Cell. Biol.* **14,** 7920–7932.
188. Walker, S. S., Reese, J. C., Apone, L. M. and Green, M. R. (1996). Transcription activation in cells lacking TAF(II)s. *Nature* **383,** 185–188.
189. White, M. J., Hirsch, J. P. and Henry, S. A. (1991). The *OPI1* gene of *Saccharomyces cerevisiae*, a negative regulator of phospholipid biosynthesis, encodes a protein containing polyglutamine tracts and a leucine zipper. *J. Biol. Chem.* **266,** 863–872.
190. Wickner, R. (1996). Double-stranded RNA viruses of *Saccharomyces cerevisiae*. *Microbiol. Rev.* **60,** 250–265.
191. Wickner, R. B. and Leibowitz, M. J. (1976). Two chromosomal genes required for killing expression in killer strains of *Saccharomyces cerevisiae*. *Genetics* **82,** 429–442.
192. Williams, F. E. and Trumbly, R. J. (1990). Characterization of *TUP1*, a mediator of glucose repression in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **10,** 6500–6511.
193. Williams, L. A. and Kane, C. M. (1996). Isolation and characterization of the *Schizosaccharomyces pombe* gene encoding transcript elongation factor TFIIS. *Yeast* **12,** 227–236.
194. Winston, F. and Carlson, M. (1992). Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. *Trends Genet.* **8,** 387–391.
195. Winston, F., Dollard, C. and Ricupero-Hovasse, S. L. (1995). Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. *Yeast* **11,** 53–55.
196. Wu, A., Wemmie, J. A., Edgington, N. P., Goebl, M., Guevara, J. L. and Moye-Rowley, W. S. (1993). Yeast bZip proteins mediate pleiotropic drug and metal resistance. *J. Biol. Chem.* **268,** 18850–18858.
197. Wu, A. L., Hallstrom, T. C. and Moye-Rowley, W. S. (1996). *ROD1*, a novel gene conferring multiple resistance phenotypes in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **271,** 2914–2920.
198. Xu, S., Falvey, D. A. and Brandriss, M. C. (1995). Roles of *URE2* and *GLN3* in the proline utilization pathway in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **15,** 2321–2330.
199. Yoon, H., Miller, S. P., Pabich, E. K. and Donahue, T. F. (1992). *SSL1*, a suppressor of a *HIS4* 5′-UTR stem-loop mutation, is essential for translation initiation and affects UV resistance in yeast. *Genes Devel.* **6,** 2463–2477.
200. Yoshida, S., Ikeda, E., Uno, I. and Mitsuzawa, H. (1992). Characterization of a staurosporine- and temperature-sensitive mutant, *stt1*, of *Saccharomyces cerevisiae*: *STT1* is allelic to *PKC1*. *Mol. Gen. Genet.* **231,** 337–344.

201. Yoshida, S., Ohya, Y., Goebl, M., Nakano, A. and Anraku, Y. (1994). A novel gene, *STT4*, encodes a phosphatidylinositol 4-kinase in the *PKC1* protein kinase pathway of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **269,** 1166–1172.
202. Yoshida, S., Ohya, Y., Hirose, R., Nakano, A. and Anraku, Y. (1995). *STT10*, a novel class-D VPS yeast gene required for osmotic integrity related to the *PKC1*/*STT1* protein kinase pathway. *Gene* **160,** 117–122.
203. Yoshida, S., Ohya, Y., Nakano, A. and Anraku, Y. (1995). *STT3*, a novel essential gene related to the *PKC1*/*STT1* protein kinase pathway, is involved in protein glycosylation in yeast. *Gene* **164,** 167–172.
204. Zhou, Z. and Elledge, S. J. (1992). Isolation of *crt* mutants constitutive for transcription of the DNA damage inducible gene *RNR3* in *Saccharomyces cerevisiae*. *Genetics* **131,** 851–866.

