Cancer therapeutics in yeast

Mary-Ann Bjornsti

Department of Molecular Pharmacology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105
Correspondence: mary-ann.bjornsti@stjude.org

The budding yeast *Saccharomyces cerevisiae* is a genetically tractable model system with which to establish the cellular target of a given agent and investigate mechanisms of drug action.

Introduction

Recent years have seen the advent of nonmammalian models (such as *Drosophila*, *C. elegans*, zebrafish, and yeast) for drug discovery and development. This has been driven in part by the completion of genome sequences and new methodologies that facilitate somatic cell genetics, such as gene knockout and RNA interference (for reviews, see Amatruda et al., 2002; Dean et al., 2001; Hudson et al., 2002; Kumar and Snyder, 2001; Paddison and Hannon, 2002). Such advances enable the manipulation of a single gene product in the context of isogenic cell systems and multicellular organisms in order to investigate drug action. The myriad genetic tools available to study wide-ranging conditional and null mutant phenotypes in haploid cells, coupled with the high degree of conservation of basic cell cycle machinery, render the yeast *Saccharomyces cerevisiae* particularly invaluable for studies of cellular pharmacology and cancer therapeutics (Forsburg, 2001; Kumar and Snyder, 2001; Ma, 2001; Reid et al., 1998; Sherman, 2002). This genetically tractable microorganism is amenable to the manipulations necessary to identify the cellular target of a chemotherapeutic agent or to decipher the pathways and processes that modulate cell sensitivity to a given agent. Drug-induced synthetic lethality in different yeast mutants can also be exploited to define specific genetic defects in cancer cells that potentiate therapy-induced cytotoxicity. Ultimately, a more thorough understanding of drug mechanism provides a conceptual framework for the clinical development of novel therapeutics and the identification of new targets for drug development.

Yeast as a model system

The haploid genome of the budding yeast *S. cerevisiae* is of relatively low complexity (1.2×10^7 bp) and is packaged into 16 well-characterized chromosomes (Cherry et al., 1997). This was the first eukaryotic genome for which the complete sequence was reported, with ~6,000 open reading frames (ORFs) predicted to encode proteins of ≥ 100 amino acid residues (Goffeau et al., 1996). The relative scarcity and limited size of introns contributes to an average gene size of 1.5 kb. Annotated information on gene and protein functions is available through several databases, including the *Saccharomyces* Genome Database (SGD), with links to databases such as Gene Ontology (GO), which provides comparative analyses of gene product functions from different model organisms, and the GRID database of genetic and physical interactions (Ball et al., 2001; Dwight et al., 2002; Issel-Tarver et al., 2002). Curated protein databases, such as the Yeast Protein Database (YPD), are also maintained as part of a collection of protein databases from other fungal and more complex eukaryotic systems that comprise the BioKnowledge Library (Csank et al., 2002).

The *Saccharomyces* Genome Deletion Project consortium has deleted over 95% of the predicted ORFs in the yeast genome with a *kanMX*4 resistance module flanked by unique 20-mer sequences (Giaever et al., 2002; Shoemaker et al., 1996). This, in essence, barcodes each gene deletion such that the under- or overrepresentation of a given deletion in pooled cultures can be assessed under various conditions by hybridization to high-density oligonucleotide arrays. About 20% of yeast genes are essential for growth in rich media and, one-third of yeast genes have been extensively characterized. Nevertheless, over 30% of yeast ORFs have no known function.

S. cerevisiae is a useful model system with which to study cellular processes and pathways that have proven intractable in higher eukaryotes. In part, the utility of this organism lies in its facile genetics and in highly conserved cell cycle machinery and metabolic pathways, with clear human orthologs (Forsburg, 2001; Kumar and Snyder, 2001; Sherman, 2002). This microorganism has the added advantage of rapid growth, as dispersed single haploid or diploid cells, where recessive mutant phenotypes may be analyzed in mitotically stable haploid strains. Also characteristic of budding yeast is relatively high rates of homologous recombination. The predominance of recombination between homologous sequences allows for the precise insertion of DNA sequences at specific locations within the yeast genome (Rothstein, 1991). In combination with PCR-based methods, it is rather trivial to replace a gene with a selectable marker or a mutant allele (Johnston et al., 2002). Similar approaches can be used to introduce regulated promoters upstream of a given ORF or epitope/GFP (green fluorescent protein) tags within a coding region (Longtine et al., 1998). This has been facilitated by the availability of a variety of selectable markers (see Table 1) such as the *URA3* gene, which restores uracil prototrophy (ability to grow on media lacking uracil) to a strain harboring a nonreverting *ura3* mutant allele.

Another aspect of yeast cell biology that facilitates the analysis of drug action is the availability of DNA vectors that can be maintained as episomal plasmids or integrated into the genome via homologous recombination (Table 1) (Christianson et al., 1992; Funk et al., 2002; Sikorski and Hieter, 1989). In low copy YCp vectors, the presence of centromeric DNA (such as *CEN6*) and an *ARS* origin of replication ensure the mitotic stability of DNA molecules that replicate once per cell cycle. In YEp vectors (20–50 copies/cell), the 2 μm origin is derived from a naturally occurring plasmid. These shuttle vectors also contain DNA elements necessary for amplification in bacteria.

If the cellular target of a drug of interest is known, then genetic screens can be designed to define events upstream or downstream of drug-target interactions that dictate cell sensitivity. If the target is unknown or the mechanism of drug action is

Table 1. Yeast nomenclature and tools[a]

Symbol	Definition
YFG1 (Your Favorite Gene)[b]	Gene (wild-type allele or dominant mutant)
ygf1	Recessive mutant allele
ygf1Δ	Deletion mutant
ygf1-1	Specific mutant allele or mutation
Ygf1p	Protein product
Marker	Selection
URA3	Media lacking uracil[c]
LEU2	Media lacking leucine
HIS3[d]	Media lacking histidine
TRP1	Media lacking tryptophan[e]
kanMX4	Kanamycin/G418 resistance
GFP[f]	None—fluoresces green when exposed to U.V. light
Promoter	Characteristics
GAL1	Regulated: glucose-repressed, galactose-induced
CUP1	Regulated: copper-inducible
MET15	Regulated: methionine-repressed
ADH	Constitutive
GPD	Strong constitutive
Vector[g]	Characteristics
YCp (centromeric plasmid) low copy number	•Centromere (*CEN*)/origin of replication (*ARS*) ensure mitotic stability •Bacterial Amp^R and ori
YEp (episomal plasmid) high copy number	•2 μm origin of replication from yeast 2 μm plasmid •Bacterial Amp^R and ori
YIp (integrating plasmid)	•Bacterial Amp^R and ori

[a] Representative examples are given for each category.
[b] Yeast genes have three-letter names that are often acronyms of descriptions of specific pathways or biological functions.
[c] *URA3* encodes orotidine-5'-phosphate decarboxylase. Negative selection: *ura3* auxotrophs are resistant to 5-FOA (5-fluoro-orotic acid).
[d] *his5*$^+$ from the fission yeast, *Schizosaccharomyces pombe*, will also complement *S. cerevisiae his3* auxotrophs.
[e] *TRP1* encodes phosphoribosylanthranilate isomerase. Negative selection: *trp1* auxotrophs are resistant to 5-FAA (5-fluoroanthranilic acid).
[f] GFP encodes green fluorescent protein. A yeast codon corrected GFP variant is used.
[g] The pRS series of centromeric, episomal, and integrating vectors enjoys wide use, with polylinker sequences flanked by T3/T7 primer sites (Christianson et al., 1992; Sikorski and Hieter, 1989).

unclear, these issues can also be approached genetically, assuming the starting yeast strain is sensitive to the drug (i.e., exhibits a 3-log drop in the number of cells forming colonies upon drug exposure). Drug inhibitory effects may be either cytotoxic or cytostatic. If conditions cannot be empirically determined that restrict cell growth in the presence of a given agent (such as alterations in media pH or the introduction of mutations that increase cell permeability), then genetic approaches may be used to induce a sensitive phenotype, which may be exploited in subsequent screens to investigate other aspects of drug action. For example, in the case of camptothecin, the target of

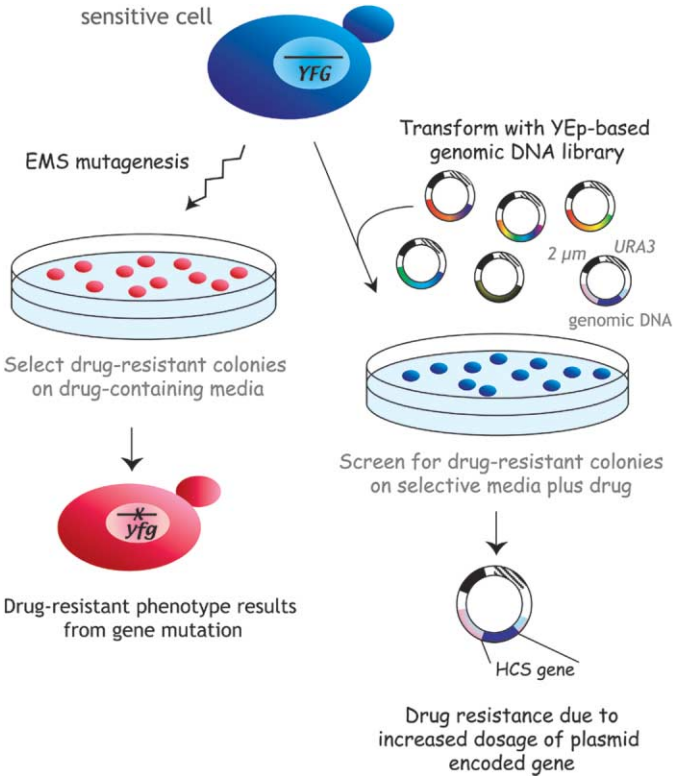

Figure 1. Classic genetic screens for drug resistance

This figure depicts independent strategies for conferring a drug-resistant phenotype in wild-type cells that are sensitive to the growth inhibitory effects of the drug. On the left, mutations that alter gene functions necessary for drug sensitivity are selected following the exposure of wild-type cells to a mutagen. The dosage suppressor screen on the right depicts the selection of drug-resistant cells following transformation of wild-type cells with a YEp-based multicopy yeast genomic DNA library. The increased expression of plasmid-encoded genes confers drug resistance.

the drug is DNA topoisomerase I. By deleting the chromosomal copy of *TOP1* and overexpressing a plasmid-borne copy of *TOP1* from the galactose-inducible promoter, the cells exhibit enhanced sensitivity to camptothecin on galactose-containing media and are resistant to the drug in the presence of dextrose (Kauh and Bjornsti, 1995; Reid et al., 1997). The increase in drug sensitivity overcomes the relative insensitivity of wild-type yeast strains to camptothecin and simplifies the elimination of trivial plasmid-borne mutations in *TOP1*.

Genetic screens to investigate drug action

Genomic approaches have been taken to define cellular responses to drug and radiation-induced DNA lesions. For example, Giaever et al. (1999) explored alterations in gene dosage as a means of identifying drug targets by assessing alterations in the growth of heterozygous deletion strains, where only one allele of a given gene was deleted. Screening the set of homozygous diploid strains deleted for nonessential genes, Resnick and colleagues defined over 100 loci that function to modulate cell sensitivity to ionizing radiation (Bennett et al., 2001). A recent comparison of homozygous diploid deletion strain survival with transcriptional responses induced by distinct classes of DNA lesions failed to establish a relationship

between increased transcript levels and gene function in mediating cell survival (Birrell et al., 2002). These findings question the utility of microarray profiling of gene expression in predicting therapeutic response. Clearly, such approaches provide important information regarding drug action; however, they are necessarily limited to nonessential genes or pathways that are sensitive to gene dosage.

This primer will focus on classical genetic screens to investigate drug action. The advantage of these approaches is the potential to isolate (1) a broad range of genetic alterations, including mutations in essential and nonessential genes that produce a gain-of-function or partial loss-of-function, and (2) conditional mutations where differences in drug sensitivity are only observed under certain circumstances, such as high temperature. Another advantage is the ability to incorporate specific genetic alterations in the parental strain used to set up the screen.

Yeast sensitivity to drugs

The relative insensitivity of yeast cells to many chemotherapeutic agents limits the utility of this system to study drug action. Drug resistance may be a consequence of poor permeability, active drug efflux, the steady state levels of a drug target, or the absence of specific activities necessary for drug activation (Kolaczkowska and Goffeau, 1999; Le Crom et al., 2002; Nitiss and Wang, 1991; Reid et al., 1998; Simon et al., 2000). In cases where drug permeability or transport is an issue, deletion of specific components of the *PDR* drug resistance pathway, such as the Pdr1p transcription factor or ATP binding cassette transporters (Pdr5p or Snq2p) (Reid et al., 1997; Simon et al., 2000), or the introduction of mutations that globally affect drug permeability (*ise1 or ISE2*) (Nitiss and Wang, 1988) can enhance the cytotoxic activity of a given agent to allow for the selection of resistant mutants. In cases where the levels of a particular drug target are at issue, regulated promoters can be introduced to alter gene expression in order to optimize cell sensitivity (as described for camptothecin above). While such genetic alterations are impractical to implement on a genomic scale in sets of deletion strains, they are easily incorporated into a parental yeast strain used in classical genetic screens.

In cases where a pathway or gene function is completely lacking in yeast, it may be possible to "humanize" yeast cells by expressing a human cDNA from a yeast promoter. However, the utility of this approach depends on the gene/pathway in question. For example, yeast has not proven useful in the study of nucleoside analogs, due to differences in nucleotide salvage and biosynthetic pathways. However, expression of Herpes simplex thymidine kinase in yeast that lack this enzyme allows for the cells to utilize exogenous thymidine or BrdU (Lengronne et al., 2001).

Another example is apoptosis, which convention dictates occurs exclusively in multicellular organisms. Conceptually, this was supported by the lack of critical components of the apoptotic apparatus—including caspases and death receptors. However, the absence of specific processes in a eukaryotic cell in which other cell cycle, repair, and checkpoint regulatory pathways are intact can also be exploited to examine protein without the confounding activities of related family members. For example, the regulated expression of human procaspases, either alone or in combination, enables a direct assessment of the hierarchical proteolytic processing and activation of specific procaspases (Kang et al., 1999). The lethal phenotype induced by certain caspases could provide the basis for a suppressor

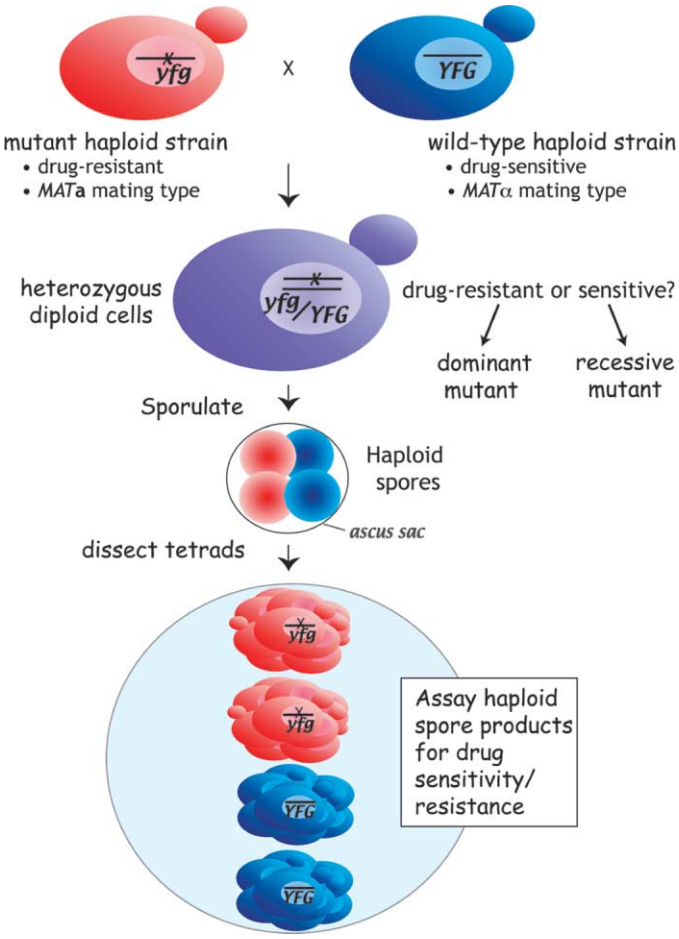

Figure 2. Characterization of drug-resistant *yfg* mutants

This figure depicts the genetic manipulations necessary to determine (1) whether a mutation in a single *YFG* gene (for *Your Favorite Gene*) is sufficient to render cells drug-resistant, or if multiple gene defects are involved, and (2) if drug resistance is dominant or recessive. The salient features are backcrossing the mutant strain with wild-type (drug-sensitive) cells of the opposite mating type and assessing the drug sensitivity of the resultant heterozygous diploid cells. The four haploid spore products of a single meiotic event are encased in an ascus sac. Following enzymatic digestion, individual spores can be gently microdissected (tetrad dissection) and germinated on rich media to form independent colonies.

screen or the selection of specific inhibitors. Moreover, recent studies indicate that a highly regulated cell death program does, in fact, occur in yeast (Jin and Reed, 2002; Madeo et al., 2002). Clearly, the design of genetic screens based on the drug-sensitive phenotype of yeast cells expressing a human cDNA must also consider the relevance of the findings to drug action in human cells.

Genetic screens: Suppressors of drug sensitivity

In the following discussions of yeast genetic screens, the focus will be limited to mechanisms of drug action and cellular processes that determine cellular response. Yeast genetics, coupled with cell biology and biochemistry, have proven invaluable in defining metabolic and repair pathways, cell cycle machinery, and checkpoint functions, and in uncovering redundant gene functions. With regards to drug action, however, the intent is to

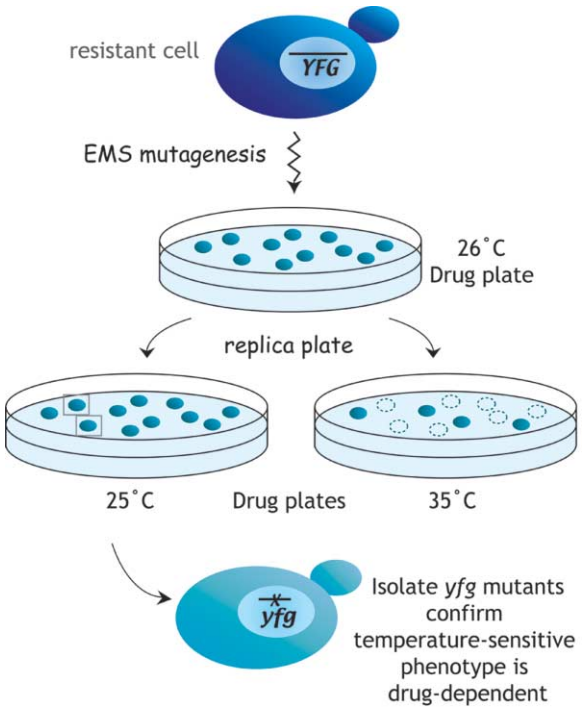

Figure 3. The isolation of mutants exhibiting conditional drug sensitivity

In this scheme, cells resistant to low concentrations of a drug are exposed to a mutagen, and colonies exhibiting temperature-sensitive cell growth in the presence of drug are isolated. The dashed lines indicate the lack of colony formation at the restrictive temperature (35°C). This screen for drug-induced synthetic lethality can also be exploited to define cold-sensitive mutants.

take advantage of well-defined pathways and/or to place novel gene functions in the context of known cellular processes.

Two classic genetic screens for drug resistance are diagrammed in Figure 1. The scheme on the right is a screen for high copy suppressors of drug sensitivity. Drug-sensitive cells are transformed with a genomic DNA library in which random fragments of yeast DNA are cloned into a YEp-based vector with a selectable *URA3* marker, and resistant colonies are selected on drug containing media. The underlying principle is that increased gene copy number of a wild-type gene (20–50 copies per cell) will augment drug resistance (Forsburg, 2001). For example, if a drug inhibits the activity of an essential rate-limiting enzyme, then increased enzyme levels would diminish drug-induced toxicity. Alternatively, an increase in repair pathways, a specific drug transporter, or activation of compensatory pathways would also yield a resistant phenotype. The advantage of this approach is that plasmid-encoded dosage suppressors can be identified by recovering the vector from individual resistant colonies and querying the database with DNA sequences obtained from each end of the genomic DNA insert. Blast searches of yeast genome databases will define the chromosome fragment contained in the plasmid, and provide information on the number and identity of putative ORFs. Subsequent subcloning and rescreening of individual ORFs will identify the high copy suppressor gene.

A *GAL1*-promoted cDNA library may also be used to selectively overexpress individual cDNAs on galactose-containing media (Liu, 2002; Measday and Hieter, 2002). The inclusion of a single cDNA in each plasmid obviates the need to subclone individual ORFs and eliminates some of the complications attendant with promoter regulation. Although both strategies require DNA libraries of high quality, the greater complexity of cDNA libraries may necessitate screening of large numbers of transformants to ensure adequate representation of gene sequences expressed at low levels.

In a second genetic approach, schematized on the left side of Figure 1, drug-sensitive haploid cells are subjected to a mutagen (such as ethyl methyl sulfonate [EMS] or U.V. light) and resistant colonies are selected on drug-containing media (Lawrence, 2002). The premise is that mutation-induced alterations in gene function enhance cellular resistance to drug-induced effects. For simplicity, the mutation here is in a gene designation *YFG* (for *Y*our *F*avorite *G*ene). As haploid cells contain one copy of each gene, recessive loss-of-function mutations as well as dominant gain-of-function mutations can be obtained. Examples include mutations in the drug target that render the enzyme resistant to inhibition (such as the targets of rapamycin, *TOR1*, and *TOR2*) (Heitman et al., 1991) or increased expression of a transcription factor that regulates the expression of a drug-specific transporter (such as a dominant *PDR1-10* mutant which induces elevated the levels of the Snq2p transporter and resistance to camptothecin) (Reid et al., 1997).

Similar strategies may be applied to mammalian cells in culture. However, the strength of the yeast system lies in the facile genetics that enable the isolation and identification of the mutant allele or dosage suppressor genes. Consider the resistant *yfg* mutant isolated in Figure 1. The genetic manipulations diagrammed in Figure 2 will determine whether the mutant is recessive or dominant and if the mutant phenotype derives from a single gene mutation. This information will then determine how the wild-type allele of *yfg* can best be isolated and characterized.

Identification of *YFG (Your Favorite Gene)*

Yeast cells exhibit highly regulated pathways of mating and sporulation, which allow the recovery of all four meiotic spore products encased in an ascus sac (Pringle and Hartwell, 1981; Sherman, 2002). As shown in Figure 2, the haploid *yfg* mutant is mated with an isogenic, unmutagenized *YFG* strain of opposite mating type. The inclusion of different selectable markers in the mutagen-treated *MAT***a** strain and the unmutagenized *MAT*α strain facilitates diploid strain selection (for instance, in a cross of *MAT***a**, *TRP1*, *his3*Δ, *yfg* cells with isogenic *MAT*α, *trp1*Δ, *HIS3*, *YFG* cells, diploids are selected on media lacking tryptophan and histidine). The heterozygous *yfg*/*YFG* diploids can be assessed for drug sensitivity to determine whether the drug-resistant phenotype is recessive or dominant. As detailed below, the recessive/dominant character of an *yfg* mutant will dictate the means of identifying the ORF that was mutated.

In either case, an analysis of the meiotic products of sporulation will determine whether the resistant phenotype is a consequence of a single gene defect or if multiple mutations are required. Obviously, the genetic analysis of single gene defects is more straightforward. The sporulation program can be initiated by nutrient deprivation, and micromanipulation allows each haploid spore to be isolated, germinated on rich media, and examined for sensitive/resistant phenotypes (Sherman, 2002). If drug resistance results from a single gene defect (or two closely linked mutations), then wild-type and mutant alleles will assort in approximately equal numbers to give a ratio of 2 resistant:2 sensitive spore products per tetrad. If, on the other hand,

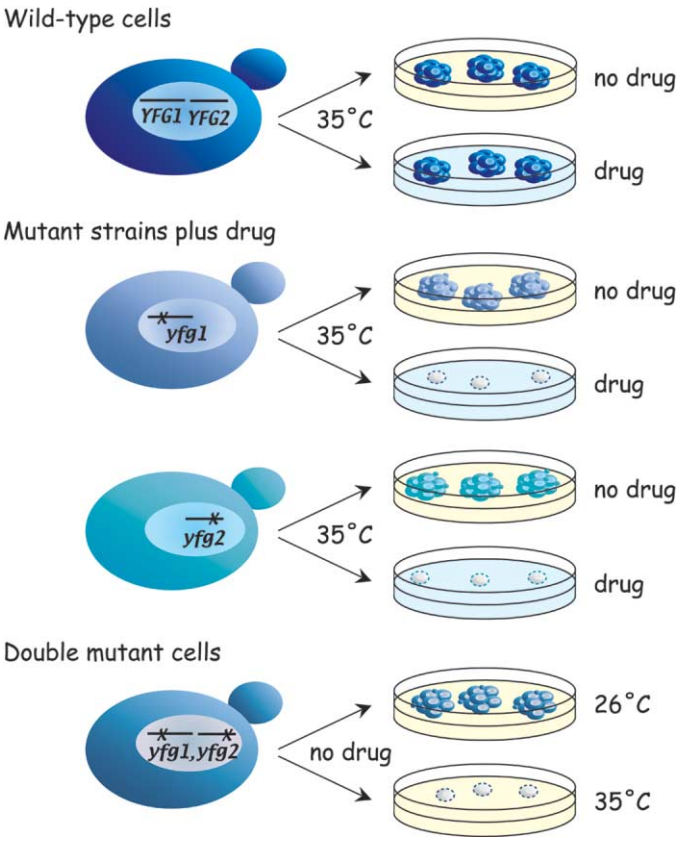

Figure 4. Synthetic lethal interactions

This figure compares the phenotypes of drug-induced synthetic lethality for *yfg1* and *yfg2* strains at 35°C in the presence of drug and the synthetic lethality induced by loss of redundant functions for the double *yfg1, yfg2* mutant at 35°C in the absence of drug. Yellow plates lack drug, while blue plates contain low concentrations of drug insufficient to inhibit wild-type cell growth at any temperature. The dashed lines indicate the lack of colony formation.

mutations in two unlinked genes are required for drug resistance, then the random assortment of the wild-type and mutant alleles will approach an overall ratio of 1 resistant:3 sensitive spore products when multiple tetrads are examined.

Once single gene defects have been defined, successive rounds of backcrossing mutant with wild-type cells followed by selection of drug-resistant haploid spore products will eliminate unrelated mutations that may affect cell growth. Since heavily mutagenized cultures are typically used in genetic screens, extensive backcrossing is critical for the subsequent characterization of *yfg* mutant phenotypes (Lawrence, 2002). If multiple recessive *yfg* mutants are isolated, complementation analysis (mating of each *yfg* mutant with another and assessing diploid cell drug resistance) will determine whether the mutations are in same gene or unlinked genes. For example, if diploid cells obtained by mating recessive mutants *yfg1* and *yfg2* exhibit a resistant phenotype, then the strains most likely harbor mutation(s) in the same gene. If the *yfg1*, *yfg2* diploid is sensitive to the drug, then two genes are likely involved. This approach can also be used to generate strains with the desired combination of genetic mutations, selectable markers, and mating type for additional studies.

For recessive *yfg* mutants, which segregate 2:2, the wild-type *YFG* allele may be cloned by complementation of the resistant phenotype (restoration of drug sensitivity). To achieve this, haploid *yfg* cells are transformed with an YCp vector-based yeast genomic library (Liu, 2002). Replica plating of the transformants onto selective media, plus or minus drug, will allow the identification of *yfg* cells that harbor a plasmid-borne copy of the wild-type *YFG* allele. Subsequent isolation of plasmid DNA from single colonies grown in the absence of drug, followed by database queries with sequences obtained from the yeast genomic DNA insert, will define the *YFG* ORF. If the *yfg* mutant exhibits other recessive phenotypes that reduce cell growth or viability under different environmental conditions (such as an inability to grow on a nonfermentable carbon source), the selection of the wild-type *YFG* allele would simply involve screening for plasmids that restore cell growth rather than inhibit colony formation.

To identify dominant *YFG* mutant alleles, a genomic YCp-based DNA library is constructed with DNA isolated from the mutant strain, which is then used to transform the recessive wild-type cells to select for drug resistance (Reid et al., 1997). Although this may seem an onerous undertaking, the construction of DNA libraries is quite straightforward (Liu, 2002), and the efficiency of selecting drug-resistant colonies was established in the initial screen. In either case, integrating a selectable marker into the *YFG* locus in a wild-type haploid strain and assessing the segregation of marker and drug resistance in backcrosses with the *yfg* mutant will confirm the genetic identity of the *YFG* clone.

If the *YFG* gene (or dosage suppressor gene) is nonessential, then targeted disruption of the chromosomal ORF will determine if gene deletion exacerbates cell sensitivity to the drug. If the gene product is well characterized, this approach can provide an entrée into specific functional pathways. Typically, more than one *yfg* mutant (or high copy suppressor) will be obtained, which may comprise different components of a given pathway or redundant functions. Alterations in cell morphology and cell cycle distribution can also provide important insights into drug action and suppressor functions.

Conditional mutants

Classic genetic screens, based on the acquisition of drug resistance, are typically restricted to nonessential gene functions and pathways affected by gene dosage. An alternative strategy that avoids the lethal consequences of null mutations in essential genes is to screen for conditional mutants, where cell viability is unaffected by drug action under one set of experimental conditions and impaired under different conditions. As diagrammed in Figure 3, this approach can be adapted to select for mutations in genes that normally protect cells from the adverse affects of drug action (Fiorani and Bjornsti, 2000; Reid et al., 1999). In the context of cancer therapeutics, the identification of gene products or pathways that function to suppress the cytotoxic/cytostatic activity of a drug would provide important insights into mechanisms regulating tumor cell response.

In this scenario, low concentrations of drug are insufficient to inhibit wild-type yeast cell growth. Following mutagenesis, viable cells are plated onto nonselective media at 25°C and subsequently replica plated onto drug-containing media at 25°C and 35°C. If the activity of a given gene product diminishes the growth inhibitory effects of the drug (confers resistance), then mutations that selectively impair gene function at 35°C would exhibit drug-dependent cell death at the higher temperature

(confer sensitivity). Rescreening for cell viability at 35°C in the absence of drug will eliminate mutants exhibiting a temperature-sensitive phenotype, independent of drug action. The spectrum of temperature sensitive mutants isolated might include loss-of-function mutations in nonessential genes, hypomorphic (reduced activity) alleles of essential genes, gain-of-function mutations, and dominant-negative mutations. A similar screen for temperature-sensitive mutants exhibiting enhanced sensitivity to sublethal levels of a DNA topoisomerase I mutant enzyme that mimics the cytotoxic action of camptothecin identified nine gene products that function to suppress camptothecin-induced cell death (Fiorani and Bjornsti, 2000; Reid et al., 1999). Six of the nine genes (including *CDC45*, *DPB11*, and *TAH11*) are essential. As mutant cell viability was not affected in the absence of camptothecin-induced DNA lesions, sufficient gene function was retained to maintain cell viability at 35°C. Nevertheless, mutation-induced alterations in protein function defined novel activities for these gene products in suppressing the cytotoxic activity of camptothecin.

Conceptually, this approach is a modified synthetic lethal screen (see below) where the combination of a nontoxic concentration of drug with a nonlethal mutation induces cell death. This suggests that a functional interaction between the gene product and the drug (or drug-induced lesion) is required for cell viability. Similar principles apply to redundant gene functions, where an essential activity is provided by one of two gene products.

Synthetic lethality

As diagrammed in Figure 4, analyses of synthetic lethal interactions constitute a powerful genetic strategy for defining functional interactions and defining pathways (Phizicky and Fields, 1995). Consider, for example, conditional *yfg1* and *yfg2* mutants isolated in the screen for drug synthetic lethality at 35°C in Figure 3. Based on the principles detailed in Figure 2, these single gene defects involve different genetic loci, *YFG1* and *YFG2*. However, when the two mutant alleles are combined in a single haploid cell, the double *yfg1, yfg2* mutant cell exhibits a temperature-sensitive phenotype in the absence of drug. This suggests that both gene products function to suppress drug-induced lethality, and that they also share a common essential function. Indeed, a similar analysis of the *cdc45-10* and *dpb11-10* mutants isolated in the aforementioned screen for mutants exhibiting enhanced sensitivity to topoisomerase I-targeted drugs uncovered a synthetic lethal phenotype of the double *cdc45-10, dpb11-10* mutant at 35°C (Reid et al., 1999). The persistent accumulation of Okazaki-sized DNA fragments in S phase suggested a common essential function in processive DNA replication that was also required for resistance to camptothecin. The function of human homologs of *CDC45* and *DBP11* in regulating tumor cell response to camptothecin is currently being pursued.

An analysis of mutant cell phenotypes, under restrictive conditions, may also be informative and provides a means of ordering gene function. For instance, if nonallelic mutations, such as *yfg1* and *yfg2*, induce distinct terminal phenotypes in the presence of drug, then the phenotype of the double *yfg1*, *yfg2* mutant can be used to ascertain whether Yfg1p acts upstream, downstream, or commensurate with Yfg2p.

Genetic interactions

Alterations in *yfg1* mutant phenotype can be exploited in secondary genetic screens to (1) isolate dosage suppressors of drug-induced *yfg1* cell death or (2) screen for compensatory mutations in genes other than *yfg1* that restore cellular resistance after EMS mutagenesis. Potential mechanisms include the stabilization of a thermolabile Yfg1 protein, an increase in compensatory or redundant repair pathways, increased expression of a transporter, or a compensatory change in protein structure that restores protein-protein interactions.

Ultimately, biochemical studies of alterations in protein function and protein-protein interactions predicted by such genetic interactions will provide a more compelling insight into drug mechanism. Nevertheless, with the principles laid out in this primer, it is possible to define the cellular target of an unknown agent and to genetically decipher the functional components of a pathway or pathways that regulate cellular responses to a given drug. These findings may have immediate impact in the clinical development of novel therapeutics or suggest novel targets for future drug development.

Acknowledgments

Many thanks to Peter Houghton for helpful comments and my apologies for any oversights in citing studies relevant to the principles presented. This work has been supported, in part, by NIH grants CA58755 and CA70406, CA21675 Cancer Center grant, and ALSAC.

References

Amatruda, J.F., Shepard, J.L., Stern, H.M., and Zon, L.I. (2002). Zebrafish as a cancer model system. Cancer Cell *1*, 229–231.

Ball, C.A., Jin, H., Sherlock, G., Weng, S., Matese, J.C., Andrada, R., Binkley, G., Dolinski, K., Dwight, S.S., Harris, M.A., et al. (2001). *Saccharomyces* Genome Database provides tools to survey gene expression and functional analysis data. Nucleic Acids Res. *29*, 80–81.

Bennett, C.B., Lewis, L.K., Karthikeyan, G., Lobachev, K.S., Jin, Y.H., Sterling, J.F., Snipe, J.R., and Resnick, M.A. (2001). Genes required for ionizing radiation resistance in yeast. Nat. Genet. *29*, 426–434.

Birrell, G.W., Brown, J.A., Wu, H.I., Giaever, G., Chu, A.M., Davis, R.W., and Brown, J.M. (2002). Transcriptional response of *Saccharomyces cerevisiae* to DNA-damaging agents does not identify the genes that protect against these agents. Proc. Natl. Acad. Sci. USA *99*, 8778–8783.

Cherry, J.M., Ball, C., Weng, S., Juvik, G., Schmidt, R., Adler, C., Dunn, B., Dwight, S., Riles, L., Mortimer, R.K., and Botstein, D. (1997). Genetic and physical maps of *Saccharomyces cerevisiae.* Nature *387*, 67–73.

Christianson, T.W., Sikorski, R.S., Dante, M., Shero, J.H., and Hieter, P. (1992). Multifunctional yeast high-copy-number shuttle vectors. Gene *110*, 119–122.

Csank, C., Costanzo, M.C., Hirschman, J., Hodges, P., Kranz, J.E., Mangan, M., O'Neill, K., Robertson, L.S., Skrzypek, M.S., Brooks, J., and Garrels, J.I. (2002). Three yeast proteome databases: YPD, PombePD, and CalPD (MycoPathPD). Methods Enzymol. *350*, 347–373.

Dean, M., Rzhetsky, A., and Allikmets, R. (2001). The human ATP-binding cassette (ABC) transporter superfamily. Genome Res. *11*, 1156–1166.

Dwight, S.S., Harris, M.A., Dolinski, K., Ball, C.A., Binkley, G., Christie, K.R., Fisk, D.G., Issel-Tarver, L., Schroeder, M., Sherlock, G., et al. (2002). *Saccharomyces* Genome Database (SGD) provides secondary gene annotation using the Gene Ontology (GO). Nucleic Acids Res. *30*, 69–72.

Fiorani, P., and Bjornsti, M.-A. (2000). Mechanisms of DNA topoisomerase I-induced cell killing in the yeast *Saccharomyces cerevisiae.* Ann. N Y Acad. Sci. *922*, 65–75.

Forsburg, S.L. (2001). The art and design of genetic screens: yeast. Nat. Rev. Genet. *2*, 659–668.

Funk, M., Niedenthal, R., Mumberg, D., Brinkmann, K., Ronicke, V., and

Henkel, T. (2002). Vector systems for heterologous expression of proteins in *Saccharomyces cerevisiae*. Methods Enzymol. *350*, 248–257.

Giaever, G., Shoemaker, D.D., Jones, T.W., Liang, H., Winzeler, E.A., Astromoff, A., and Davis, R.W. (1999). Genomic profiling of drug sensitivities via induced haploinsufficiency. Nat. Genet. *21*, 278–283.

Giaever, G., Chu, A.M., Ni, L., Connelly, C., Riles, L., Veronneau, S., Dow, S., Lucau-Danila, A., Anderson, K., Andre, B., et al. (2002). Functional profiling of the *Saccharomyces cerevisiae* genome. Nature *418*, 387–391.

Goffeau, A., Barrell, B.G., Bussey, H., Davis, R.W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J.D., Jacq, C., Johnston, M., et al. (1996). Life with 6000 genes. Science *274*, 563–567.

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.

Hudson, D.F., Morrison, C., Ruchaud, S., and Earnshaw, W.C. (2002). Reverse genetics of essential genes in tissue-culture cells: 'dead cells talking'. Trends Cell Biol. *12*, 281–287.

Issel-Tarver, L., Christie, K.R., Dolinski, K., Andrada, R., Balakrishnan, R., Ball, C.A., Binkley, G., Dong, S., Dwight, S.S., Fisk, D.G., et al. (2002). *Saccharomyces* Genome Database. Methods Enzymol. *350*, 329–346.

Jin, C., and Reed, J.C. (2002). Yeast and apoptosis. Nat. Rev. Mol. Cell Biol. *3*, 453–459.

Johnston, M., Riles, L., and Hegemann, J.H. (2002). Gene disruption. Methods Enzymol. *350*, 290–315.

Kang, J.J., Schaber, M.D., Srinivasula, S.M., Alnemri, E.S., Litwack, G., Hall, D.J., and Bjornsti, M.-A. (1999). Cascades of mammalian caspase activation in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. *274*, 3189–3198.

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.

Kolaczkowska, A., and Goffeau, A. (1999). Regulation of pleiotropic drug resistance in yeast. Drug Resist Update *2*, 403–414.

Kumar, A., and Snyder, M. (2001). Emerging technologies in yeast genomics. Nat. Rev. Genet. *2*, 302–312.

Lawrence, C.W. (2002). Classical mutagenesis techniques. Methods Enzymol. *350*, 189–199.

Le Crom, S., Devaux, F., Marc, P., Zhang, X., Moye-Rowley, W.S., and Jacq, C. (2002). New insights into the pleiotropic drug resistance network from genome-wide characterization of the YRR1 transcription factor regulation system. Mol. Cell. Biol. *22*, 2642–2649.

Lengronne, A., Pasero, P., Bensimon, A., and Schwob, E. (2001). Monitoring S phase progression globally and locally using BrdU incorporation in TK(+) yeast strains. Nucleic Acids Res. *29*, 1433–1442.

Liu, H. (2002). Constructing yeast libraries. Methods Enzymol. *350*, 72–86.

Longtine, M.S., McKenzie, A., 3rd, Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J.R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast *14*, 953–961.

Ma, D. (2001). Applications of yeast in drug discovery. Prog. Drug Res. *57*, 117–162.

Madeo, F., Engelhardt, S., Herker, E., Lehmann, N., Maldener, C., Proksch, A., Wissing, S., and Frohlich, K.U. (2002). Apoptosis in yeast: a new model system with applications in cell biology and medicine. Curr. Genet. *41*, 208–216.

Measday, V., and Hieter, P. (2002). Synthetic dosage lethality. Methods Enzymol. *350*, 316–326.

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.

Nitiss, J.L., and Wang, J.C. (1991). Yeast as a genetic system in the dissection of the mechanism of cell killing by topoisomerase-targeting anticancer drugs. In DNA Topoisomerases in Cancer, M. Potmesil and K.W. Kohn, eds.(Oxford: Oxford University Press), pp. 77–92.

Paddison, P.J., and Hannon, G.J. (2002). RNA interference: the new somatic cell genetics? Cancer Cell *2*, 17–23.

Phizicky, E.M., and Fields, S. (1995). Protein-protein interactions: methods for detection and analysis. Microbiol. Rev. *59*, 94–123.

Pringle, J.R., and Hartwell, L.H. (1981). The *Saccharomyces cerevisiae* cell cycle. In The Molecular Biology of the Yeast *Saccharomyces*, J.N. Strathern, E.W. Jones, and J.R. Broach, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory), pp. 97–142.

Reid, R.J., Fiorani, P., Sugawara, M., and Bjornsti, M.-A. (1999). CDC45 and DPB11 are required for processive DNA replication and resistance to DNA topoisomerase I-mediated DNA damage. Proc. Natl. Acad. Sci. USA *96*, 11440–11445.

Reid, R.J.D., Kauh, E.A., and Bjornsti, M.-A. (1997). Camptothecin sensitivity is mediated by the pleiotropic drug resistance network in yeast. J. Biol. Chem. *272*, 12091–12099.

Reid, R.J.D., Benedetti, P., and Bjornsti, M.-A. (1998). Yeast as a model organism for studying the actions of DNA topoisomerase-targeted drugs. Biochim. Biophys. Acta *1400*, 289–300.

Rothstein, R. (1991). Targeting, disruption, replacement, and allele rescue: Integrative DNA transformation in yeast. Methods Enzymol. *194*, 281–301.

Sherman, F. (2002). Getting started with yeast. Methods Enzymol. *350*, 3–41.

Shoemaker, D.D., Lashkari, D.A., Morris, D., Mittmann, M., and Davis, R.W. (1996). Quantitative phenotypic analysis of yeast deletion mutants using a highly parallel molecular bar-coding strategy. Nat. Genet. *14*, 450–456.

Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19–27.

Simon, J.A., Szankasi, P., Nguyen, D.K., Ludlow, C., Dunstan, H.M., Roberts, C.J., Jensen, E.L., Hartwell, L.H., and Friend, S.H. (2000). Differential toxicities of anticancer agents among DNA repair and checkpoint mutants of *Saccharomyces cerevisiae*. Cancer Res. *60*, 328–333.