Minireview

A cultivated taste for yeast

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Abstract

The availability of complete genomic sequences of *Saccharomyces cerevisiae* has catalyzed a cultural change in the practice of yeast biology, providing opportunities to develop high throughput techniques to define protein function, to define drug targets, and to discover and characterize drugs.

For decades, yeast biology has been dually justified as an effort to understand fully the cellular processes of a group of single-celled eukaryotes and for its potential to lead to advances in human health. Sequence-based trees of life provide compelling evidence that plants, animals and fungi - once considered three primary kingdoms in a five-kingdom tree - are closely related branches within the eukaryotic domain [1]. It is well known that homologs of proteins first identified for key roles in yeast cell-cycle control [2], secretion [3] and pro-hormone processing [4] play similar roles in humans. Furthermore, similarities between fungal, animal and plant cells have created challenges in developing fungal-directed antibiotics that are not toxic to hosts. Although yeast biology has illuminated many processes in yeast and human cells by homology, the yeast cell has also become a laboratory of heterology. In the 1980s, heterologous use of yeast typically referred to protein production [5]. In the 1990s, legions of researchers were introduced to yeast two-hybrid systems to identify binding partners of proteins expressed in yeast [6]. Although it is too early to predict the leading heterologous use of yeast of the next decade, screens and selections to identify small molecules that act on proteins expressed in yeast hold tremendous promise [7]. The power of combinatorial chemistry to screen huge numbers of drugs can now be multiplied by the power of yeast genetics to assay genetic variation. At a recent Camrbidge Healthtech Institute meeting in Miami entitled 'Exploiting Yeast Molecular Biology for Therapeutics' (January 20–21, 2000), some of the latest homologous and heterologous uses of yeast biology were presented, along with new methods for studying yeast biology that became conceivable in the post-genomic era.

Given a substrate, find the enzyme

Thousands of yeast genes and their corresponding products were identified by classical genetic approaches that began with identification of mutant phenotype and progressed 'forward' to the gene and product. Thousands of other genes were discovered by 'reverse' genetic approaches, in which mutants were obtained last. The original reverse genetics experiments were fractionation-based; one purified a protein of interest, sequenced it, and then cloned and disrupted the corresponding gene (for example, see [8]). More recently, reverse genetic approaches have been driven by identification of homologous sequences (for example, see [9-11]). The availability of complete genomic information has made possible a new type of reverse genetics not driven by sequence similarity but rather based on a novel fractionation scheme.

For example, when Mark Martzen, Elizabeth Grayhack and Eric Phizicky at the University of Rochester set out to identify an enzyme, they began in the classical manner by developing an assay to screen for activity. Rather than fractionating a large, single yeast lysate to identify the enzyme by biochemical means, these researchers used a set of yeast strains carrying fusions of glutathione-S-transferase (GST) to 6,080 open reading frames (ORFs) [12]. The GST fusions, developed in the laboratory of Stanley Fields at the

University of Washington [13], were grown in 64 pools, each containing 95 ORF-GST fusions plus one control GST construct. To identify the gene responsible for an enzyme activity of interest, Martzen and colleagues first screened the 64 pools to identify which one contained the activity. Because each pool originated from constructs held in a 96 well plate, they then screened the 8 rows and 12 columns from the identified plate. The single point of intersection of pooled rows and pooled columns defines the single ORF responsible for an activity. The first publication from the Martzen group using this technique described three enzymes identified in this way [12], and the group's biochemical genomics scoreboard now includes a DNA-binding protein and numbers 12 additional enzymes, several of which carry out RNA splicing reactions.

Given an enzyme, validate it as a drug target

In humans, elevated serum homocysteine concentration is associated with vascular, skeletal and neurological disorders. The most common genetic basis for clinical presentation of severe hyperhomocysteinemia is cystathionine β-synthase (*CBS*) deficiency, found in 1 child in about 300,000 [14]. Moreover, levels of homocysteine vary in populations and there is strong epidemiological linkage between elevated total homocysteine levels and vascular disease and stroke [15]. Whereas 50% of *CBS*-deficient patients respond to high doses of vitamin B6, and vitamin B6 can also reduce total homocysteine in the majority of the human population, a specific drug that could boost Cbs enzyme activity in *CBS*-deficients and in those at risk for stroke might improve the quality of life for many people. Warren Kruger of the Fox Chase Cancer Center has developed a simple yeast-based assay to phenotype human *CBS* genes and has validated mutant forms of Cbs as targets that could be remedied by drugs. Wild-type human *CBS* coding sequences complement the cysteine deficiency of *S. cerevisiae cys4* deletion mutants, and loss-of-function alleles of human *CBS* fail to complement *cys4* [16]. In the course of cloning new *CBS* alleles, Kruger's laboratory amplified a patient's *CBS* genes and recovered a large number of yeast transformants that were cys^- and two that were CYS^+. The two CYS^+ isolates contained PCR-induced intragenic suppressor mutations of the patient's loss-of-function allele. Both mutations resulted in truncation of an inhibitory carboxy-terminal domain. Nearly all *CBS* patients carry missense mutations and nearly all of these alleles are suppressed by deletion of the carboxy-terminal domain [17]. At the Miami meeting, Kruger presented data showing that a variety of point mutations in the carboxy-terminus suppress the hypomorphic phenotypes of *CBS* mutations and that carboxy-terminal suppressors are not allele-specific. Thus, chemical inactivation of the carboxy-terminal domain of Cbs should be achievable and should boost the function of low-activity enzymes. Furthermore, the combinatorial chemist has a choice of primary screens: increase mutant Cbs enzyme activity *in vitro* or generate prototrophy in a *cys4* yeast strain carrying mutant human *CBS*.

Given a target, find a drug

Kathleen Young of Wyeth-Ayerst Inc. spoke about her group's efforts to find 'disinactivators' of a human potassium channel, Kv1.1/Kvβ1. Inactivation of such channels, mediated by an interaction between the S4-S5 cytoplasmic linker domain of the α subunit and the amino-terminal inactivation ball of the β subunit [18] might be associated with seizures and hippocampal ischemia [19]. For this reason, small molecules that block the association of specific α and β subunits are considered to have therapeutic potential. As demonstrated earlier with calcium channel fragments [7], two-hybrid interactions can be constructed to produce growth inhibition such that drugs that block the interaction restore growth. The new screen for potassium channel disinactivators involved more than 176,000 compounds and has apparently identified a compound, WAY-349, with *in vitro* efficacy and specificity for the Kv1.1/Kvβ1 channel.

Given a drug, find the target

Although protein-target-directed screening has become the predominant way to identify small molecules today, the majority of approved drugs were identified on the basis of animal or cellular assays. In fact, most of the classical compounds that owe their roots to botanicals and other natural products were originally identified by human self-medication. Thus, it should come as no surprise that a great many drugs have modes of action that are not understood. John Phillips and his colleagues at Rosetta Inpharmatics Inc. use two genomic methods to clarify the mode of action of drugs. First, they have developed a complete set of promoter fusions to the green fluorescent protein of *Aequoria victoria* that allows them to monitor the effect of a small molecule on the expression of each yeast gene [20]. Administration of a drug to yeast strains transformed with the set of reporter constructs provides a fluorescent profile of the genes that become activated or repressed in response to a drug. In the best cases, such as that of *erg11*, the ergosterol-starved gene expression profile of *erg11* mutant cells is indistinguishable from that of cells treated with the antifungal drug clotrimazole. Second, they test the drug sensitivity [21] of a large and growing set of strains that are heterozygous for individual open reading frames [22,23]. Because each gene disruption strain carries two unique 20 nucleotide 'barcodes', deletion mutants can be maintained in a pool [22,23]. Upon drug administration, the relative viability of each deletion strain can be assessed by amplification of barcodes from the pooled strains using fluorescently-labeled PCR primers and array hybridization to a DNA chip containing the barcode oligonucleotide probes [21]. Particular deletion mutants that are more sensitive to a drug than the majority of deletion mutants produce a less fluorescent signal upon drug administration and are identifiable by losses of particular barcode signals. The most drug-sensitive strains are those in which the gene products targeted by the drugs have been rendered heterozygous [21]. This method is complementary to the classical method of cloning the target

by virtue of multicopy-plasmid-dependent resistance [24]. The advantage of the new method is that it is highly parallel. New compounds can be profiled with ease, and can be used to identify not only primary and secondary targets via sensitivity [25] but, additionally, loss-of-function mutations that result in drug-resistance.

Given a pathogenic fungus, find a drug target

Candida species are among the most common fungal pathogens to humans. *C. albicans*, which is asexual and diploid, is refractory to 'forward' or classical genetics though *C. glabrata* is a haploid and, thus, conditionally essential genes or genes essential for adhesion to human epithelial cells can be identified by forward mutagenesis and screening. Having developed methods for homologous and nonhomologous integrative transformation in *C. glabrata* [26], Brendan Cormack (Johns Hopkins University) selected 96 40mer oligonucleotide barcodes and integrated them into the disrupted *ura3* locus of a *C. glabrata* strain. Each of these barcoded strains was then subjected to random insertional mutagenesis with the *S. cerevisiae URA3* gene and 100 independent URA$^+$ integrants were frozen away for each barcoded strain [27]. In each strain, the URA3 insertion is expected to have affected a different gene. By picking one transformant from each of the 96 barcoded transformations, Cormack was able to assemble 100 pools each containing an independent *URA3* insertion in a uniquely identifiable strain.

Cormack has already described a screen in which 50 of the pools, representing as many as 4,800 independent *C. glabrata* mutants, were passaged through an adherence assay on human cultured epithelial cells. By probing a DNA 'macrochip' (a nylon filter the size of a 96 well plate on which the 96 bar-code oligonucleotides were spotted) with radioactively labeled barcode PCR reactions from *C. glabrata* cells that grew on human cells, Cormack identified 16 mutants that failed to adhere. Of these, 14 contained insertions in the same gene, named *EPA1* for epithelial adhesin, a calcium-dependent lectin with a consensus sequence for addition of a glycosyl-phosphatidylinositol anchor [27]. Cormack also presented data showing that pools of barcoded mutant *C. glabrata* strains can be passaged through a mouse in the primary mutant hunt: 96 mutants being screened per animal. Cormack's approach of barcoding the cells to be mutagenized rather than linking the barcode to the mutants makes his barcodes reusable and independent of specialized array hybridization equipment.

Though the yeast genome has been complete for four years, the majority of yeast gene functions are poorly characterized. Many of the approaches presented in Miami have the potential to assign functions to significant portions of the yeast proteome. As knowledge of yeast cell biology expands, one can expect greater light to be reflected on all eukaryotes and further cultivation of yeast for pharmacological applications.

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