# Budding Yeast for Budding Geneticists: A Primer on the *Saccharomyces cerevisiae* Model System

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**SUMMARY** The budding yeast *Saccharomyces cerevisiae* is a powerful model organism for studying fundamental aspects of eukaryotic cell biology. This Primer article presents a brief historical perspective on the emergence of this organism as a premier experimental system over the course of the past century. An overview of the central features of the *S. cerevisiae* genome, including the nature of its genetic elements and general organization, is also provided. Some of the most common experimental tools and resources available to yeast geneticists are presented in a way designed to engage and challenge undergraduate and graduate students eager to learn more about the experimental amenability of budding yeast. Finally, a discussion of several major discoveries derived from yeast studies highlights the far-reaching impact that the yeast system has had and will continue to have on our understanding of a variety of cellular processes relevant to all eukaryotes, including humans.

THE model organism *Saccharomyces cerevisiae* is commonly known as baker's yeast or brewer's yeast. Indeed, the scientific name *cerevisiae* derives from old world terminology for beer (Mortimer 2000). To scientists, *S. cerevisiae* is classified as a fungus or mold, although those who enjoy artisan breads or a great microbrew would probably rather not think of it that way! *S. cerevisiae* is a single-celled eukaryote, and thus it contains membrane-bound organelles, such as a nucleus, endomembrane system, and mitochondria. Yeast cells divide as rapidly as once every 90 min under optimal laboratory conditions, through a process of budding in which smaller daughter cells pinch, or bud, off the mother cell (see Figure 1). The common name "budding yeast" derives from this notable feature of cell division and distinguishes *S. cerevisiae* from the fission yeast, *Schizosaccharomyces pombe*, also a powerful model organism. *S. cerevisiae* cells in nature switch readily between two mating types: haploid **a** cells mate with haploid α cells to form diploids. Under nutrient-poor conditions, diploids can be induced to undergo meiosis and sporulation, forming four haploid spores, two of each mating type (Figure 2). Due to their microscopic size and simple growth requirements, yeast cells are inexpensive and easy to grow in the laboratory. Unbudded yeast cells are ~5 μm in diameter, between bacteria and human cells in size. They form colonies on agar plates in the laboratory in a few days with no special incubators required. Yeast stocks are maintained by freezing at −80° in glycerol or can be freeze-dried and stored at room temperature for years. In nature, yeasts are found in abundance in vineyards, but can also be found associated with oak trees and in other natural habitats (Greig and Leu 2009). However, it is unclear whether *S. cerevisiae* as a species occurs naturally or exists solely as a domesticated species. We note that recent genomic analyses suggest that wild populations of *S. cerevisiae* exist (Mortimer 2000; Greig and Leu 2009).

The countless domesticated strains of *S. cerevisiae* are recognized the world over for their ability to ferment sugars to ethanol and carbon dioxide, producing a variety of beverages enjoyed by all cultures. Human use of yeast is thought to have begun >7000 years ago with the discovery that crushed grapes in water would ferment, as yeast is found in high abundance on the fruit (Mortimer 2000). Yeast can be purposefully added to other sources of sugars, such as grains, malts, or other plant materials, to produce other alcoholic beverages. Although a relatively "newer" discovery, the carbon dioxide released by yeast has been used for centuries to raise bread dough, resulting in the light texture of leavened bread. For most of history, wine makers relied solely on the yeast present on the harvested grapes, but brewers and bakers would lovingly transfer their starter cultures from batch to batch as *S. cerevisiae* worked unknown and unseen to ferment the various sugars provided


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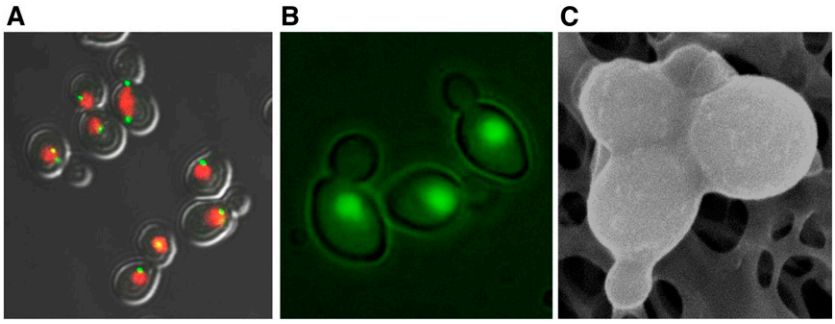


**Figure 1** Budding yeast cells. (A) Confocal fluorescence microscopy of haploid yeast expressing Spc42-GFP (green: spindle pole body marker) and Histone H2-mCherry (red: nuclear marker). The yeast strain was constructed by Shawnecca Burke, an undergraduate in M.E.M.'s research laboratory by crossing strains kindly provided by Jan Skotheim (Stanford University) and Mark Winey (University of Colorado at Boulder) (photo by Mary Miller). (B) Epifluorescence microscopy of diploid yeast cells expressing Spt16-GFP, a nuclear protein. The yeast strain was generated by students in A.A.D.'s Spring 2005 Advanced Cell Biology class (photo by Andrea Duina). (C) Electron microscopy of dividing yeast cells (photo by Christine Walls). Note that buds are visible emerging from some of the cells in each of the panels. Unbudded cells are ~5 μm in diameter.

by their owners. In the late 1800s, the Carlsberg Laboratory introduced the brewing industry to the process of science, and Emil Christian Hansen discovered how to purify yeast from mixed starter cultures (Greig and Leu 2009). In the past 60 years, a rather short time evolutionarily speaking, the strains of *S. cerevisiae* used in modern baking, brewing, fermenting, and wine-making have been carefully cultured, selected, and purified by these respective industries; each strain has its own particular characteristics and they are not interchangeable (Mortimer 2000). However, a few wineries continue the practice of indigenous yeast fermentation, relying on the naturally provided yeasts of the grapes.

Yeast research has certainly established for itself a place in history, beginning with the famed first demonstrations of enzymatic activities outside of a living cell by Edward Buchner and continued with work with yeast extracts through the early 1900s, which laid the groundwork for biochemical analysis of metabolic pathways (Bohley and Fröhlich 1997). In the 1930s, Øjvind Winge and Carl Lindegren began the first work on yeast as an experimental organism (Mortimer and Johnston 1986; Mortimer 2000). Winge started with strains isolated by Hansen at the Carlsberg brewery laboratory, while Lindegren used strain EM93 (isolated by Emil Mrak from rotting figs in California). Strain EM93 was amenable to genetic crosses, a key development in modern yeast genetics. Multiple generations of genetic crosses allowed researchers to isolate specific traits and eventually to characterize the genes that encode those traits. One of the most widely used experimental strains, S288C, was constructed in the early 1950s by Robert Mortimer (primarily from EM93) and was subsequently used as the parental strain for the isolation of biochemical mutants (Mortimer and Johnston 1986). Whereas the yeast system was beginning to attract attention from the scientific community, most notably through brilliant genetic and biochemical experiments by Fred Sherman in the 1960s and 1970s (nicely reviewed in Liebman and Haber 2013), there was little to distinguish it from the dozens of experimental organisms being developed at the time. Then in 1978 *S. cerevisiae* was successfully transformed with a plasmid that had been replicated in the bacterium *Escherichia coli*. The publication of the "Transformation of yeast" launched the stellar research career of this simple organism (Hinnen *et al.* 1978).

What ultimately has distinguished yeast as a premier model organism is the ease with which researchers can move genes in and out of yeast cells, either on plasmids or within the yeast chromosomes. Thus, geneticists can easily mutate or manipulate yeast gene expression and study the resulting phenotypic effects. Following the discovery of transformation, the subsequent rapid development of a veritable menu of replicating plasmids and selectable markers set in place the "awesome power of yeast genetics." Naturally occurring yeast replication origins were adapted to create plasmids that replicate within the yeast cell. The cloning of selectable markers into these plasmids, in the form of auxotrophic marker genes that provide essential enzymes needed for growth or drug resistance genes, led to the development of large sets of standardized plasmids. Moreover, in the absence of a replication signal, transformed DNA integrates readily into the yeast genome via homologous recombination, allowing for targeted gene sequences to be disrupted. The advent of polymerase chain reaction (PCR), along with the efficiency of homologous recombination in yeast, has led to the development of "designer deletion strains," which, combined with the comprehensively annotated *Saccharomyces* Genome Database (SGD; http://www.yeastgenome.org/). gives yeast geneticists flexibility in experimental design that is the envy of investigators working with other model organisms (Goffeau *et al.* 1996; Brachmann *et al.* 1998; Cherry *et al.* 2012).

## *S. cerevisiae* Genome Organization

### *The genes*

In 1996, the *S. cerevisiae* genome became the first fully sequenced eukaryotic genome (Goffeau *et al.* 1996). In addition to representing a landmark achievement in the history of eukaryotic biology, the sequencing of the *S. cerevisiae* genome has provided a wealth of information on various aspects of genome organization and evolution. In a typical haploid budding yeast cell, the ~12,000 kb of genomic DNA are

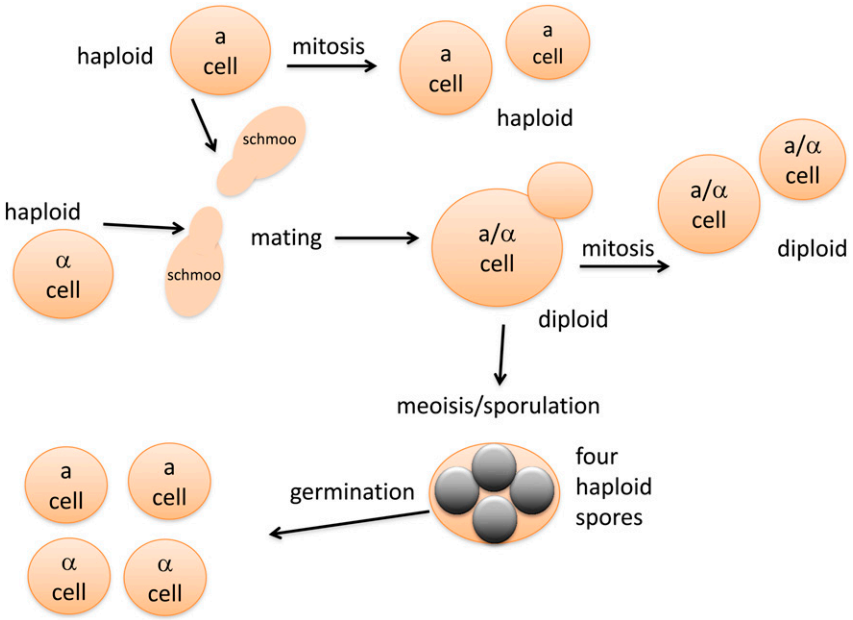


**Figure 2** A simplified life cycle diagram of laboratory budding yeast. Haploid yeast cells can be one of two mating types: *MAT***a** (**a** cell) or *MAT*α (α cell). These cells can undergo mitotic cell division through budding, producing daughter cells. In laboratory strains, the mating type of haploid cells is stable due to the absence of a functional *HO* endonuclease. The two cell types release pheromones, initiating the formation of schmoos and subsequent mating, resulting ultimately in a stable diploid *MAT***a**/*MAT*α (**a**/α cell). Diploid cells also divide mitotically by budding to produce genetically identical daughter cells. Under nitrogen-poor conditions, diploids are induced to undergo meiosis, forming four haploid spores, which can germinate into two *MAT***a** cells and two *MAT*α cells.

subdivided into 16 chromosomes, thought to have arisen following an ancient whole-genome duplication event from an ancestral set of 8 distinct chromosomes (Kellis *et al.* 2004). As would be expected for any eukaryotic genome, the budding yeast genome is studded with a large number of genes that can be broadly grouped into those that encode proteins (protein-coding genes) and those that do not (noncoding genes). According to the SGD (http://www.yeastgenome.org/), as of February 3, 2014, the number of "verified open reading frames (ORFs)" in the reference strain S288C stood at 5076. Perhaps surprisingly for such a well-characterized and extensively studied model system, there are still a relatively large number of ORFs—745 as of the same date—likely to contain information for the synthesis of proteins but for which experimental data are lacking as to whether the proteins are in fact expressed in cells and, if so, what their functions might be. The density of protein-encoding genes in the budding yeast genome is quite high (one gene every ~2 kb), ~50-fold higher than the gene density in the human genome. High gene density is partly explained by the relatively low number of intron-containing genes in *S. cerevisiae*, estimated to be at ~4% of all genes (Goffeau *et al.* 1996; Spingola *et al.* 1999). The *S. cerevisiae* genome also harbors 786 so-called "dubious ORFs," which, while technically being ORFs, are unlikely to encode proteins.

The noncoding genes present in the budding yeast genome include those transcribed into RNA molecules involved in the translation process [transfer RNA (tRNA) genes, close to 300 in number, and ribosomal DNA (rDNA) genes, present in a tandem array of 100–200 copies on chromosome XII, encoding four distinct rRNA molecules]; those involved in pre-messenger RNA splicing (small nuclear RNA genes, 5 in total); and those that facilitate chemical modifications of a variety of RNA molecules (small nucleolar RNA genes, 76 genes in total) (Piekna-Przybylska *et al.* 2007; Kavanaugh and Dietrich 2009). Additional types of noncoding genes include *SCR1* and *TLC1*, whose RNA products participate in protein targeting to the endoplasmic reticulum and in synthesis of telomeric DNA, respectively (Singer and Gottschling 1994; Van Nues and Brown 2004), and the regulatory genes *SRG1*, *ICR1*, and *PWR1*, whose transcription directly regulates the expression of adjacent genes through a phenomenon referred to as transcription interference (Martens *et al.* 2004; Bumgarner *et al.* 2009). Additional genomic elements have been discovered that are able to regulate transcription of nearby genes through the generation of noncoding RNAs (*e.g.*, see Hongay *et al.* 2006; Houseley *et al.* 2008; Pinskaya *et al.* 2009; Gelfand *et al.* 2011; van Werven *et al.* 2012; Castelnuovo *et al.* 2013), and, based on the finding that noncoding RNA synthesis appears to be a widespread phenomenon in budding yeast (David *et al.* 2006; Neil *et al.* 2009; Xu *et al.* 2009), many more such regulatory examples are likely to be identified in the future.

### *Genomic regions involved in chromosome maintenance*

Several features of the yeast genome ensure that chromosomes are properly replicated, maintained, and eventually segregated to cells following either mitosis or meiosis. Origins of replication are located at ~20- to 40-kb intervals on each chromosome. Many of these elements were originally identified as sequences of a few hundred base pairs that confer plasmids (otherwise devoid of their own replication origin) the ability to replicate in budding yeast cells following transformation experiments and were thus coined as autonomously replicating sequences (ARSs). Key functional aspects of ARS elements include the ability to recruit a variety of factors involved in triggering DNA replication (with the origin recognition complex being a critical one) and the intrinsic propensity to easily unwind to facilitate the DNA replication process (for a review, see Dhar *et al.* 2012). Whereas many ARSs are *bona fide* origins of replication in

their natural chromosomal contexts, some are not, thus necessitating the use of sophisticated techniques to unequivocally designate a specific ARS as a true chromosomal origin of replication. The latest information on ARS designations in the *S. cerevisiae* genome comes in the form of a website called OriDB (http://cerevisiae.oridb.org/; also see Nieduszynski *et al.* 2007; Siow *et al.* 2012).

Proper chromosome segregation during mitosis and meiosis relies on the ability of kinetochore microtubules to make specific contacts with chromosomes. Each of the 16 *S. cerevisiae* chromosomes contains a centromere to direct assembly of the kinetochore, itself responsible for making direct contacts with microtubules. These "point" centromeres are unusual among eukaryotes in that they are very short (~125 bp) and are not surrounded by heterochromatin. Nevertheless, because of their simplicity and their conserved functions, budding yeast centromeres have served as an important model for understanding eukaryotic centromere biology (for review, see Mehta *et al.* 2010). Combining centromere (CEN) and ARS sequences in artificial plasmids allows researchers an additional level of control of plasmid copy number in the yeast system.

The ends of chromosomes are capped by telomeres, composed of specialized DNA sequences and associated proteins. A typical *S. cerevisiae* telomeric region includes a heterogeneous stretch of ~300 bp of the $C_{1\text{-}3}A$ /$TG_{1\text{-}3}$ repeat and an adjacent subtelomeric region referred to as the telomere-associated sequence. Telomeres are one of three regions in the budding yeast genome that form heterochromatin-like environments—the others being the rDNA locus and the silent mating-type cassettes—and as such they have been used extensively as a model for understanding heterochromatin structure and function (for reviews, see Buhler and Gasser 2009; Wellinger and Zakian 2012).

### *Additional genetic elements found in the S. cerevisiae genome*

The *S. cerevisiae* genome houses a large number of so-called long terminal repeat (LTR) retrotransposons, called *Ty* elements, scattered across all 16 chromosomes. The active forms of these transposable elements can transpose within the genome through a cycle that, similar to mammalian retroviruses, includes transcription and translation of the element and subsequent assembly of viral-like particles (VLPs). Reverse transcription of the retrotransposons' RNA into complementary DNA (cDNA) by reverse transcriptase occurs within the VLPs and is followed by insertion of the cDNA into the genome. A recent survey of the S288C genome identified 483 *Ty* elements, 427 of which are inactive solo LTRs (Carr *et al.* 2012). Interestingly, *Ty* elements have evolved mechanisms of targeted integration, favoring integration near Pol III-transcribed genes (*e.g.*, tRNA genes) or telomeres and silent mating-type cassettes—likely as a strategy to maximize their chances of survival since the targeted regions are gene-poor and are therefore more likely to withstand integration events without detrimental effects to the host cell (for review, see Boeke and Devine 1998).

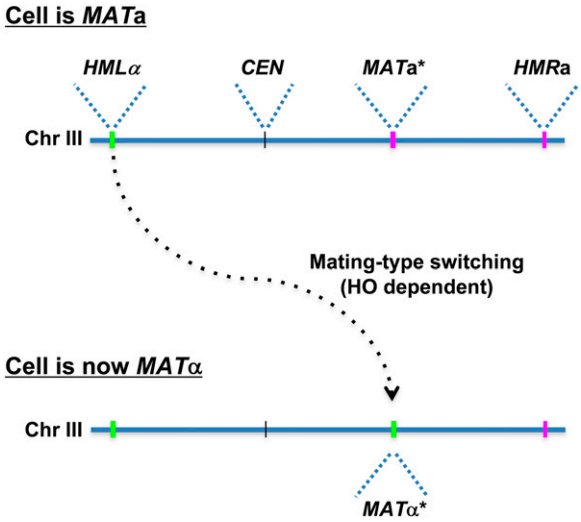


**Figure 3** Cartoon representation of the *S. cerevisiae* chromosome III and simplified view of the change that it undergoes following a mating-type switching event. In a haploid *MAT***a** cell, the *MAT* locus on chromosome III houses the *MAT***a** allele (top). During a mating-type switching event, the genetic information at *HML*α is used to replace the *MAT***a** allele at the *MAT* locus with the *MAT*α allele. The resulting chromosome III (bottom) expresses *MAT*α information, which causes the cell to become phenotypically *MAT*α. A similar mating-type switching mechanism operates during the switch of *MAT*α cells into *MAT***a** cells. The genetic elements shown in the diagram (*HML*α, *CEN*, *MAT***a**, *MAT*α, and *HMR***a**) and their relative positions across the chromosome are depicted roughly to scale (note that chromosome III is 316,620 bp in length). The asterisks next to *MAT***a** and *MAT*α highlight the fact that these alleles are actively expressed, as opposed to the alleles present at the *HML*α and *HMR***a** loci that are transcriptionally silent.

Chromosome III in *S. cerevisiae* harbors genetic information that determines the mating-type identity of cells. Haploid yeast cells can be either mating type **a** or α (*MAT***a** and *MAT*α, respectively), which, under the appropriate conditions, can mate with each other to generate *MAT***a**/*MAT*α diploids. These diploid cells cannot mate but can reproduce mitotically or can undergo meiosis to produce haploid spores (Figure 2). The mating behavior of yeast cells is determined by the *MAT* locus housed on the right arm of chromosome III (Figure 3). This locus can harbor one of two nonhomologous alleles called *MAT***a** and *MAT*α: *MAT***a** cells contain the *MAT***a** allele, *MAT*α cells contain the *MAT*α allele, and, in most cases, diploid cells contain both alleles (each located on one of the two homologous chromosome IIIs). The two *MAT* alleles express different sets of proteins, which, through a rather complex mechanism, regulate proper mating-type behavior of cells (reviewed in Haber 2012). Chromosome III also carries an additional copy of each *MAT* allele at two additional loci: the *HMR***a** locus located near the end of the right arm of the chromosome contains the *MAT***a** allele and the *HML*α locus located near the end of the left arm of the chromosome contains the *MAT*α allele. Unlike the *MAT* locus, however, *HMR***a** and *HML*α (also known as silent mating-type cassettes) are embedded in heterochromatin and are therefore transcriptionally

**Table 1 Summary of Kill-It study**

| Experimental goal | Technique/resource used | Result |
|---|---|---|
| Isolate yeast mutants resistant to Kill-It | Selection of spontaneous mutants | |
| Identify complementation groups | Mating of *MAT***a** and *MAT*α recessive mutants | |
| Clone wild-type version of the *KIR1* gene | Functional complementation | |
| Determine the sequence of *KIR1* and its chromosomal location | Sequence the library clone; BLAST | |
| Determine effect of deleting the *KIR1* gene | Targeted disruption to construct a *kir1Δ* strain | |
| Determine cellular location of Kir1 protein | Tag Kir1 at N and C termini with GFP | |
| Determine which cellular components interact with Kir1 | SGD interactions resources | |
| Determine if and where Kir1 interacts with chromatin | ChIP-seq | |
| Determine impact of a *KIR1* deletion on the expression of other genes | RNA-seq | |

silenced, but can be used by haploid cells to switch from one mating type to the other. During mating-type switching, a particular allele found at the *MAT* locus is replaced by the opposite *MAT* allele using the information located at either the *HMR***a** or the *HML*α locus (Figure 3). Commonly used laboratory strains are unable to switch mating types due to the lack of a functional form of an enzyme, the *HO* endonuclease, required to initiate the process, thus allowing for the generation and maintenance of genetically stable cell populations. The budding yeast mating-type system has served as a remarkable model system for understanding a wide array of basic cellular processes, including heterochromatin formation and maintenance, transcriptional silencing, and homologous recombination.

### *Chromosome organization within the budding yeast nucleus*

The availability of powerful genetic and cell microscopy tools, combined with the development of the chromosome conformation capture technique, which allows investigators to assess the conformation of chromosomes in live cells (Dekker *et al.* 2002), has made *S. cerevisiae* a prime model system for examining the relationship between chromosomal spatial arrangement and the regulation of processes that occur across chromosomes. Key principles obtained from current work include the notion that chromosomes adopt nonrandom positions within budding yeast nuclei [we now have a map showing how all 16 chromosomes are arranged within budding yeast nuclei (Duan *et al.* 2010)] and that specific subnuclear locations are associated with specific chromosomal processes—for example, certain inducible genes are seen to associate with the nuclear pores following their activation (reviewed in Taddei and Gasser 2012). Yet another breakthrough that features yeast is the development of a genome-wide map of nucleosome positions at base-pair resolution, representing an important step in investigating how nucleosomes drive the folding of chromosomes in living cells (Brogaard *et al.* 2012). Given its experimental tractability, *S. cerevisiae* will continue to be a major workhorse in investigations of the relationship between higher-order chromosome configurations and chromosome-based processes.

## Experimental Toolkit and Related Resources

One of the authors often jokes with students that the ease of experimental manipulation offered by *S. cerevisiae* could make one think that it is not a naturally occurring organism but that it exists only for the pleasure of those interested in understanding the intricate workings of eukaryotic cells. Although this comment may draw an occasional perplexed look, it reflects a sentiment felt by many yeast geneticists. To illustrate many of the tools and resources available to researchers who use budding yeast as an experimental organism, below we describe a hypothetical scenario and a series of experimental approaches that a budding yeast researcher (the reader in this example) might use to conduct a scientific investigation. To further promote the learning process, we have included an activity at the end of this section in which we invite readers to synthesize what they have learned to articulate a possible model describing the biological process under investigation and to envision possible future research avenues (refer to Table 1).

Imagine that, after several years of intense work as a graduate student, you have identified a new chemical compound, which we shall call "Kill-It," that appears to be highly toxic to yeast cells but that does not harm mammalian cells. You are very excited about this discovery since, given its apparent specificity toward yeast cells, Kill-It may represent a promising new antifungal drug. However, in other experiments, you have discovered that yeast cells can develop resistance to this drug. You now wish to determine the molecular mechanism that underlies this resistance. In defining the basis of resistance, you will further the understanding of the mechanism of action of the drug, influence treatment design aimed at potentially synergistic drugs, and possibly influence target-based drug design of antifungal agents in the future. The experimental strategies and hypothetical results for this project are presented below.

### *Isolation of mutants*

As a way to determine the mechanism by which yeast can develop resistance to Kill-It, you start by isolating mutant *S. cerevisiae* cells that can withstand exposure to the drug. There are several tools at your disposal to isolate such mutants. You can carry out a genetic selection experiment and transfer billions of mitotically dividing cells to solid growth medium

**Table 2 Gene and protein nomenclature for *S. cerevisiae***

| Name | Description | Nomenclature |
|---|---|---|
| YGL264W | Systematic ORF designation for the gene | Each ORF in the yeast genome is assigned a systematic name using the following conventions: Y stands for yeast; G represents the chromosome number (where A corresponds to chromosome I; B to chromosome II, etc.). This ORF is therefore located on chromosome VII; L indicates that the ORF is on the left arm of the chromosome; 264 indicates that the ORF is the 264th from the centromere, and W denotes that the coding strand of the ORF is on the Watson strand, which is the strand whose 5′ end is located at the left telomere (the complementary strand is referred to as the Crick strand, or C). |
| *KIR1*$^{+}$ | Wild-type gene | Three italicized uppercase letters and number followed by a superscripted + sign |
| *KIR1* | Dominant allele of the gene. This nomenclature is also often used to refer to the wild-type gene. | Three italicized uppercase letters and number |
| *KIR1-1*, *KIR1-2*, *etc.* | Distinct dominant alleles of the gene | Dominant mutant allele designation followed by a hyphen and a number to indicate specific allele |
| *kir1* | Recessive allele of the gene | Three italicized lowercase letters and number |
| *kir1-1*, *kir1-2*, *etc.* | Distinct recessive alleles of the gene | Recessive mutant allele designation followed by a hyphen and a number to indicate specific allele |
| *kir1*Δ | Deletion allele of the gene | Recessive mutant allele designation followed by a Δ symbol |
| Kir1p | Protein product of the gene | Three letters, with the first being uppercase, followed by a number and lower case p; all in roman font |
| Kir1 | Alternative nomenclature for protein product of the gene | Three letters, with the first being uppercase, followed by a number; all in roman font |

The gene identified in *Experimental Toolkit and Related Resources* is used as an example.

containing Kill-It and select for cells able to grow. Such cells would have acquired one or more spontaneous mutations at some point during their growth prior to exposure to Kill-It that rendered them resistant to Kill-It. If desired, the rate of mutagenesis can be increased dramatically through the use of mutagens, such as ethyl methanesulfonate. Alternatively, you could identify mutants of interest through the use of genetic screens. For these experiments, colonies or patches of haploid cells, each derived from a single cell carrying an independent pre-existing mutation, can be grown on permissive solid medium and then transferred to Kill-It-containing medium to screen for those mutants able to grow in the presence of the drug. To facilitate your studies, you could take advantage of one of the several budding yeast deletion libraries generated by the *Saccharomyces* Genome Deletion Project consortium (http://www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html), a collection of conditional mutants (Li *et al.* 2011), or a set of mutants generated by insertional mutagenesis (see Vidan and Snyder 2001).

### *Classical genetic analyses of mutants*

Using a spontaneous selection experiment, you isolate 40 mutants that form colonies in the presence of Kill-It; you call these mutants "*kir*" for *K*ill-*I*t *r*esistance. You can now take advantage of aspects of *S. cerevisiae* that make it such an effective experimental organism. First, you can easily set up genetic crosses between the *kir* mutants and wild-type cells to obtain diploids heterozygous for the *kir* mutations to determine if the mutations are recessive or dominant. This information will be important in your efforts to identify the gene in question and also provides perspective on potential mechanisms of the drug resistance that you observed in your original mutant. For example, a dominant mutation might somehow inactivate the effect of a drug while a recessive mutation might represent a target of the drug. Next, since you cleverly set up the original selection experiment using both *MAT***a** and *MAT*α cells, you have some *MAT***a** *kir* mutants and some *MAT*α *kir* mutants. By analyzing results of genetic crosses between recessive *kir* mutants, you can carry out complementation tests that will help you determine how many different genes are represented in your mutant strains. You learn that all of the 40 original mutants are recessive and that they can be grouped into a total of five complementation groups. In other experiments, you establish that in each case a single genetic mutation is responsible for the Kill-It-resistance phenotype. Thus, the mutants you have isolated define mutations in five distinct genes, which you temporarily name *KIR1-KIR5* (refer to Table 2 for gene nomenclature in *S. cerevisiae*).

### *Tools for gene identification and initial functional characterization*

You decide to focus your questions on *KIR1*. For example, what is the identity of the *KIR1* gene? Has *KIR1* already been studied by others, or are you the first person to identify it? Is there any information about the possible function of *KIR1*? On which chromosome does *KIR1* reside? To start to address these questions, you investigate whether *KIR1* is involved in morphological or cell cycle processes in the cell. You are able to make use of simple microscopic analysis of yeast containing the *kir1* mutation, making note of any changes in cell shape or cell cycle distribution (budding is coincident with

cell cycle progression, and therefore defects in cell cycle regulation can be initially characterized by this observation). You find that the *kir1* mutation does not impact cellular morphology since *kir1* mutant cells look similar to wild-type cells.

You then design an experiment to identify the *KIR1* gene using a functional complementation approach of the recessive *kir1* allele. For these experiments, you obtain a genomic DNA library derived from a wild-type strain. These libraries normally contain random pieces of wild-type yeast genomic DNA inserted into yeast centromeric plasmids, which carry a centromere and an ARS element and thus function as minichromosomes. Each plasmid in the library contains a piece of wild-type DNA that is large enough to carry several genes, and the collection of all library plasmids generally covers the entire genome multiple times. You introduce the plasmids into *kir1* cells using one of several transformation protocols, which in budding yeast are highly efficient (for examples, see Kawai *et al.* 2010). To select for *kir1* cells that contain a library plasmid following transformation, you plate the cells onto medium on which only cells that contain a plasmid can grow. The resulting transformants are then replica-plated to medium containing Kill-It to identify those that have been restored to a normal phenotype, *i.e.*, are now Kill-It sensitive, as these will likely contain a plasmid containing the wild-type *KIR1* gene. The library plasmids from Kill-it-sensitive colonies can then be easily retrieved using one of several plasmid recovery techniques (Robzyk and Kassir 1992) and sequenced. Following analysis of the genomic library fragment, you obtain the DNA sequence corresponding to the wild-type version of the *KIR1* gene. With decreasing genomic sequencing costs, it is becoming more common to bypass functional complementation and instead locate the site of a mutation by directly sequencing the genome of the mutant strain. A drawback of this genome-sequencing approach, however, is that in many instances it may be difficult to identify the mutation responsible for the phenotype under investigation if additional unrelated mutations are also present in the genome of the mutant strain.

With the DNA sequence of *KIR1* in hand, you now move to the treasure trove of information in the SGD (http://www.yeastgenome.org/; also see Cherry *et al.* 2012). As a repository for the *S. cerevisiae* genome sequence (specifically, the S288C reference strain), SGD contains an enormous amount of information on gene and protein function, and it disseminates news relevant to budding yeast researchers. You begin by using the BLAST function, which compares the *KIR1* sequence that you obtained to the entire yeast genome sequence. The BLAST analysis reveals that *KIR1* corresponds to the gene with the systematic name YGL264W. [Each gene, whether previously studied or not, is assigned a systematic name following certain guidelines (see Table 2)]. When you read about YGL264W on SGD, you are excited to find that its function has not yet been discovered! You might be the first investigator to discover a function associated with this gene. Using the Gene Registry function at SGD you are now able to "reserve" *KIR1* as the standard name for YGL264W, which will become its official standard name once you publish it in a scientific journal. (Note: YGL264W does not exist; it has been created just for this example.)

### *Computational approaches for investigating protein function*

As a way to explore the possible function of the Kir1 protein in cells (see Table 2 for protein nomenclature), you use the BLAST function available at the National Center for Biotechnology Information website (http://blast.ncbi.nlm.nih.gov/) to compare the Kir1 protein sequence against protein databases from other organisms to see if a protein similar in sequence to Kir1 has been previously characterized by others. To your delight, you find that Kir1 has substantial sequence similarities to a protein in the fruit fly *Drosophila melanogaster* that is known to be a transcription factor. SGD also enables cross-organism sequence comparisons of either proteins or DNA to identify sequences similar to *KIR1* in different *S. cerevisiae* strains (http://www.yeastgenome.org/cgi-bin/FUNGI/alignment.pl) or in other fungi (http://www.yeastgenome.org/cgi-bin/FUNGI/showAlign). Although such comparisons are not likely to provide insight into the function of Kir1, they can highlight evolutionarily conserved features of a gene or protein and thus help in the characterization of the gene and protein under investigation.

### *Genetic approaches for investigating protein function*

Many genetic tools are available in yeast to help you investigate the function of a previously uncharacterized protein. You decide to start by determining the effects of a complete knockout of *KIR1* on cell function using a one-step gene replacement approach (Figure 4A). To do this, you transform a PCR product harboring a selectable marker flanked by DNA sequences homologous to genomic regions flanking *KIR1* into wild-type diploid cells and plate the cells onto selective medium using a standard protocol (for an example, see Brachmann *et al.* 1998). The transformation is done in diploid cells so that the resulting cells remain alive even if a deletion of *KIR1* (*kir1*Δ) were to be lethal. To examine the effects of *kir1Δ*, you then induce the diploid cells to undergo meiosis, resulting in the generation of four meiotic products (two wild type and two *kir1*Δ) per original diploid cell, forming what yeast researchers call a "tetrad" (Figure 4B). The resulting tetrads can then be dissected onto solid growth medium using a light microscope equipped with a micromanipulator and the spores allowed to germinate into visible colonies (Figure 4B), which can be subsequently analyzed for specific phenotypes using a procedure commonly referred to as tetrad analysis. Based on your tetrad analysis, you find that haploid *kir1*Δ cells are able to grow and, similarly to the mutants you isolated in the initial selection experiment, they are also resistant to Kill-It (Figure 4B). Thus, *KIR1* is not a gene that is essential for the life of a yeast cell. In addition, you have ruled out the possibility that resistance to Kill-It is caused by a cryptic, secondary

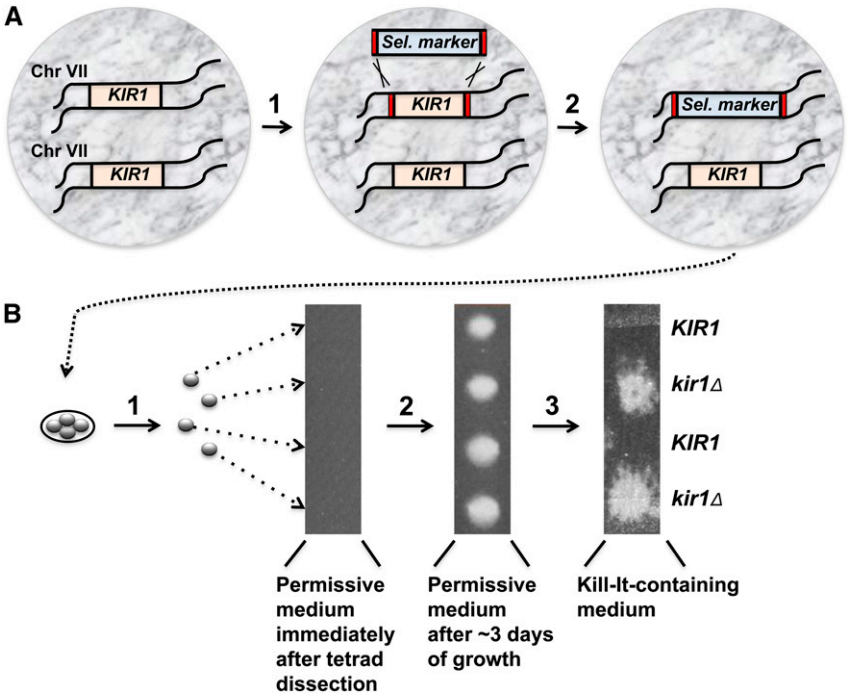


**Figure 4** One-step gene replacement and analysis of meiotic products through tetrad analysis. (A) Deletion of *KIR1*. Step 1: A *KIR1* homozygous diploid cell (*KIR1/KIR1*: only the cell nucleus is shown here) is transformed with a linear DNA molecule (usually generated by PCR) containing a selectable marker gene flanked by regions identical in sequence to those that flank the endogenous *KIR1* gene (red regions in the center panel). Step 2: Following homologous recombination between the introduced DNA fragment and one of the two *KIR1* genes, the transformed cell is heterozygous for the *KIR1* gene and its genotype is *KIR1/kir1*Δ. (B) Generation of spores, tetrad manipulation, and tetrad analysis. The *KIR1/kir1*Δ cell from A is then triggered to undergo meiosis through nitrogen starvation to produce a tetrad—a set of four spores encased in an ascus sac. Step 1: The ascus membrane is partially digested and, through the use of a light microscope equipped with a micromanipulator, the four spores are released and placed in a row onto permissive solid growth medium and allowed to germinate; note that no cells are visible to the naked eye immediately after this manipulation on the growth medium (the dark rectangle is a photograph of a section of a growth plate as it would look after the tetrad dissection). Step 2: Following ~3 days of incubation at 30°, the germinated spores give rise to visible colonies on the growth medium (the photograph shows actual yeast colonies derived from germinated spores). Step 3: The colonies are then replica-plated to solid growth medium containing Kill-It and allowed to incubate at 30° for 2 days. The 2:2 growth pattern of the colonies on the drug plate (two alive and two unable to grow) is consistent with classic Mendelian segregation of a heterozygous trait and can be used to infer the genotypes of the cells in each colony (and, by extension, of the original spores) as indicated to the right of the photograph. (Given the hypothetical nature of the experiment, it should be noted that the actual genotypes of the cells photographed in this figure are not as indicated in the figure and that the medium in the last photograph does not contain Kill-It.)

mutation in your original studies instead of by your *kir1* mutation. A plethora of additional phenotypes can also be easily scored to gain insight into the nature of the *kir1*Δ mutants (see Hampsey 1997). These and some of the experiments described in the earlier sections underscore how the ability to easily interconvert *S. cerevisiae* cells between the haploid and diploid states can be extremely helpful when carrying out genetic analyses.

The budding yeast system is also particularly well suited for a battery of genetic experiments that can uncover genetic interactions between a gene of interest and other genes. These genetic interactions often reflect physical and/or functional interactions between proteins and hence can be critically important when investigating the function of an uncharacterized protein. Examples of such genetic approaches include the isolation of spontaneous suppressor mutations and high-copy suppressors (for an extended discussion on suppression analysis in yeast, see Prelich 1999) and the implementation of synthetic lethal screens. Suppression and synthetic lethal interactions, as well as other types of genetic interactions, can also be unveiled through the use of a high-throughput methodology known as Synthetic Gene Array (SGA) analysis (for a detailed description, see Tong and Boone 2006). In an example of an SGA experiment, a query haploid strain harboring a null mutation in a gene of interest is crossed to a haploid deletion library (consisting of ~5000 haploid strains, each with a deletion of a single nonessential gene) of the opposite mating type. Using a series of replica-plating steps, haploid cells carrying the original null mutation and one of each of the ~5000 gene deletions represented in the library can be selected and assayed for suppression or synthetic-lethal interactions. Whereas these experiments can be carried out manually, investigators often make use of robots to handle the hundreds of plates required for analysis of thousands of mutants. The phenotypic data are then assembled into gene interaction networks that can be accessed as interactive maps. You instead opt to proceed using the approaches described below.

### *Determining the cellular localization of a budding yeast protein*

The function of a protein in the cell is intimately related to its subcellular localization. To investigate the localization of a protein within the cell, yeast geneticists often rely on the efficient homologous recombination system of *S. cerevisiae* to generate gene fusions between a gene of interest and the gene encoding green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*. The resulting engineered gene would then be expected to express a protein consisting of the protein of interest fused to GFP, which can be visualized in live cells using fluorescence microscopy (see Figure 1, panels A and B). You may decide to use this approach to generate two Kir1-GFP fusion proteins—one in which GFP is fused to the N terminus of Kir1 and another in which GFP is fused to the C terminus—to guard against the possibility

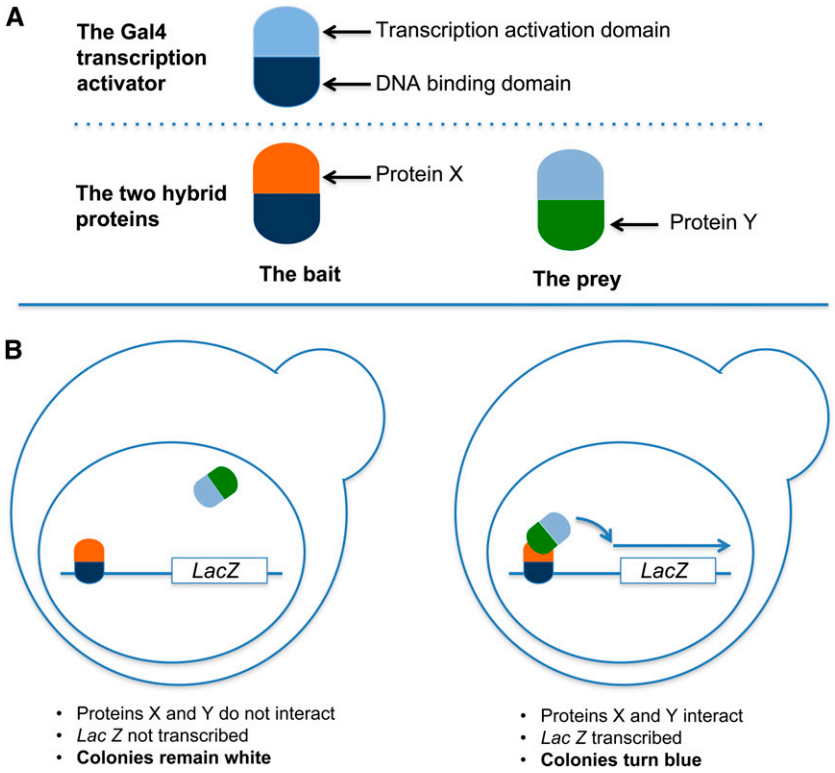


**Figure 5** The yeast two-hybrid system. (A) (Top) Representation of the yeast Gal4 transcription activator with the DNA-binding and transcription activation domains colored in different shades of blue, as indicated. (Bottom) Representations of two hypothetical hybrid proteins. The bait consists of a fusion of the Gal4 DNA-binding domain and protein X (orange), and the prey consists of the Gal4 activation domain fused to protein Y (green). (B) (Left) Hypothetical scenario in which proteins X and Y do not interact with each other. In this case, the bait protein is recruited to the regulatory region of a reporter gene (*lacZ*) but is unable to activate transcription without an activation domain. Yeast colonies derived from such cells remain white when grown in the presence of X-gal, a substrate for the *lacZ* product. (Right) Hypothetical scenario in which proteins X and Y physically interact with each other. The bait, bound to the regulatory region of the reporter gene, recruits the prey through an interaction between proteins X and Y, which in turn activates *lacZ* transcription through its activation domain. Colonies derived from these cells will turn blue when grown in the presence of X-gal. Thus, interaction between proteins X and Y can easily be tested by monitoring yeast colony color. Note that interactions between bait and prey proteins may not necessarily be direct but may be mediated by bridging proteins. Since proteins X and Y can be derived from any source, interactions between proteins from any species may be assessed using this system.

that one fusion protein may misfold and thus be potentially degraded (Longtine *et al.* 1998). However, it would be wise for you to first visit the Yeast GFP Fusion Localization Database (http://yeastgfp.yeastgenome.org/), which contains searchable protein localization data from a genome-wide study in which all budding yeast ORFs have been fused to GFP (Huh *et al.* 2003). You may be able to answer your question without doing a single experiment! Your results suggest that Kir1 is a nuclear protein, a finding consistent with its orthology to a fly transcription factor. However, the Kir1-GFP fusion protein may not function like the wild-type Kir1 protein and may not localize to its normal subcellular location. Thus, it is critical to determine whether or not the GFP-tagged version of your protein remains functional. To test the functionality of Kir1-GFP, you check haploid cells expressing the fusion protein for Kill-It sensitivity and you breathe a big sigh of relief when you see that the cells are still sensitive to Kill-It. Thus, the Kir1-GFP fusion protein has normal function, at least in relation to growth on Kill-It, and your characterized localization pattern is likely to be physiologically relevant to your studies.

### *Tools available to yeast researchers to detect protein–protein interactions*

Knowing which other proteins physically interact with Kir1 can shed light on its function. Yeast biologists have several experimental techniques at their disposal to investigate protein–protein interactions. One powerful approach is provided by the yeast two-hybrid system, which allows for the identification and the analyses of protein–protein interactions in an *in vivo* setting (Figure 5 and Chien *et al.* 1991). Global yeast two-hybrid analyses have been carried out for the budding yeast proteome and have provided a wealth of information regarding protein–protein interaction networks at a global scale (Uetz *et al.* 2000; Ito *et al.* 2001; Yu *et al.* 2008). Standard biochemical coprecipitation experiments in which investigators generate fusions between a protein of interest and an affinity tag (again taking advantage of efficient homologous recombination in yeast) and analyze interacting proteins using affinity purification followed by mass spectrometry are also widely used approaches for studying protein–protein interactions. A popular affinity tag used for such experiments is the so-called Tandem Affinity Purification (TAP) tag (Puig *et al.* 2001), and libraries containing full sets of budding yeast proteins fused to this tag have been generated (*e.g.*, Ghaemmaghami *et al.* 2003). These techniques have been applied to the yeast proteome using high-throughput technologies, generating complex networks of putative protein, RNA, and genetic interactions. Because summary results of these studies can be accessed at SGD, you (again) may not have to do any experiments yourself to find more information about Kir1! Sure enough, you check the interactions data summary at SGD and find that Kir1 appears to interact with components of Swi/Snf, a chromatin-remodeling complex often involved in activation of transcription, once again pointing to the possibility that Kir1 is involved in transcriptional regulation.

### *Wrapping-up your project using additional techniques commonly used by yeast geneticists*

The results you have obtained so far suggest that Kir1 may be a transcription factor involved in regulation of gene

**Table 3 Some resources available to yeast geneticists**

| Name | Website/company/reference | Resource |
|---|---|---|
| *Saccharomyces* Genome Database | http://www.yeastgenome.org/ (Cherry *et al.* 2012) | Gene annotations, data from high-throughput screens, and publications with links to other databases and sequence information |
| Yeast deletion project | http://www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html (Brachmann *et al.* 1998; Winzeler *et al.* 1999) | Yeast community cooperative project to systematically delete each nonessential ORF |
| Yeast deletion collection | Thermo Scientific | Yeast strains with a deletion of a specified ORF can be purchased as individual strains or as a collection |
| Designer deletion strains | Thermo Scientific (Brachmann *et al.* 1998) | Set of strains developed for efficient one-step gene replacement using common yeast markers |
| pRS plasmid collection | American Type Culture Collection (Sikorski and Hieter 1989) (Christianson *et al.* 1992) | Low-copy (CEN), high-copy (2-μm), and integrating plasmids containing common yeast markers |
| Yeast GFP-tagged ORFs | Life Technologies (Huh *et al.* 2003) | Yeast strains containing individual ORFs fused to GFP at the C terminus |
| Yeast TAP-tagged ORFs | Thermo scientific (Ghaemmaghami *et al.* 2003) | Yeast strains containing individual ORFs fused to a TAP tag at the C terminus |
| Yeast GFP Fusion Localization Database | http://yeastgfp.yeastgenome.org/ | Database of ORF–GFP fusions with images searchable by ORF name or cellular location |
| *S. cerevisiae* strain sequence alignment | http://www.yeastgenome.org/cgi-bin/FUNGI/alignment.pl | Bioinformatics tool for aligning sequences within *S. cerevisiae* strains |
| Yeast sequence strain alignment | http://www.yeastgenome.org/cgi-bin/FUNGI/showAlign | Bioinformatics tool for aligning sequences among yeast species |
| DNA Replication Origin Database | http://cerevisiae.oridb.org/ (Nieduszynski *et al.* 2007; Siow *et al.* 2012) | Bioinformatics tool for searching location of ARS across the yeast genome |

expression. To explore this possibility further, you decide to use a high-throughput version of the chromatin immunoprecipitation (ChIP) technique (known as ChIP-Seq) to interrogate if and where Kir1 physically interacts with chromosomes and the RNA-Seq technique to determine which genes, if any, are either repressed or activated by Kir1. Using these approaches, you find that Kir1 interacts with the regulatory region of a gene encoding a previously studied amino acid transporter and that the expression of this gene is greatly reduced in cells deleted for *KIR1*.

### *What have you learned about the ability of budding yeast cells to develop resistance to Kill-It?*

The hypothetical project on Kill-It has served as a convenient platform to discuss many of the tools and resources (summarized in Table 3) available to the yeast geneticist. We now invite students of yeast genetics to trace your way back to the beginning of this discussion and contemplate what you have learned about Kill-It using the different experimental approaches described. After filling in the "Result" column in Table 1, can you come up with a model that can explain the relationship you have uncovered between Kir1 function and Kill-It toxicity? What types of testable hypotheses can be formulated based on your model? What additional experiments could be designed to deepen your understanding of Kir1 function in the cell? How would you go about investigating the functions of the other Kir proteins you identified? Imagine Kir2 is localized to the plasma membrane; how could this fit in your model? There are other genetic tools to use to investigate gene and protein function in yeast. Can you think of how you could incorporate an experimental procedure not discussed here in your quest to understand the biology of Kill-It and the Kir proteins?

## Notable Advances from Research on Budding Yeast

Understanding the genetic, biochemical, cytological, and genomic approaches available in the budding yeast system is exciting, but how has this type of work advanced our understanding of living systems more generally? A quick glance at both the Lasker Awards and Nobel Prizes of the past 15 years gives examples of some especially notable work in budding yeast that has advanced our understanding of highly conserved basic cellular processes. In a brilliant series of experiments, Lee Hartwell blended basic phenotypic observations with classic genetic approaches to describe the foundations of regulated cell division in budding yeast and was awarded a Lasker Award in 1998 and a Nobel Prize in 2001. Hartwell combined morphological observations with the use of temperature-sensitive mutants to distinguish mutations that impact cellular growth from mutations in genes that regulate cell division. He was able to order events in critical pathways and initiated decades of work that established the ordered cell division cycle in both budding yeast and other eukaryotes, including humans (Hartwell *et al.* 1970; Hartwell 1992; Weinert and Hartwell 1993; Hartwell and Kastan 1994; Weinert *et al.* 1994; Paulovich and Hartwell 1995). Budding yeast continues to propel our understanding of regulated cell division: critical studies in the structure and

**Table 4 Some landmark discoveries in yeast**

| Topic of discoveries | Year | Award | Investigators |
|---|---|---|---|
| Regulated cell division | 1998 | Albert Lasker Basic Medical Research Award | Lee Hartwell, Yoshio Masui, and Paul Nurse |
| Regulated cell division | 2001 | The Nobel Prize in Physiology or Medicine | Lee Hartwell, Tim Hunt, and Paul Nurse |
| Vesicle trafficking | 2002 | Albert Lasker Basic Medical Research Award | James Rothman and Randy Schekman |
| Vesicle trafficking | 2013 | The Nobel Prize in Physiology or Medicine | James Rothman, Randy Schekman, and Thomas Südhof |
| Transcription | 2006 | The Nobel Prize in Chemistry | Roger Kornberg |
| Telomeres | 2006 | Albert Lasker Basic Medical Research Award | Elizabeth Blackburn, Carol Greider, and Jack Szostak |
| Telomeres | 2009 | The Nobel Prize in Physiology or Medicine | Elizabeth Blackburn, Carol Greider, and Jack Szostak |
| Chaperones | 2011 | Albert Lasker Basic Medical Research Award | Franz-Ulrich Hartl and Arthur Horwich |

regulation of signal transduction pathways (Mok *et al.* 2011), subcellular organization (Taddei and Gasser 2012), movement of key regulators (D'Amours and Amon 2004), checkpoint regulation [of DNA repair, spindle function, cellular growth, nutrient response, and stress (Yasutis and Kozminski 2013)], and aging (Longo *et al.* 2012) all touch on our understanding of regulated cell division and make heavy use of budding yeast as a model for more complex eukaryotic systems. This work requires integration of the many components of the yeast toolkit from genetic interaction analysis to genome-wide protein localization studies.

Another excellent set of discoveries in budding yeast that took full advantage of the yeast toolkit is Randy Schekman's work on eukaryotic vesicle trafficking (Lasker Award in 2002 and Nobel Prize in 2013). Schekman established an ordered secretory system and clarified the striking underlying mechanism in this system (Novick and Schekman 1979; Novick *et al.* 1980; Deshaies and Schekman 1987; Baker *et al.* 1988; Barlowe *et al.* 1994; Matsuoka *et al.* 1998). This work serves as the foundation of applications that allow production and secretion of medically significant molecules such as insulin from yeast and has impacted a wide variety of fields ranging from neurobiology to virology (Hou *et al.* 2012).

Our understanding of other basic cellular processes has also been significantly shaped by the budding yeast toolkit. Roger Kornberg (awarded a Nobel Prize in 2006) deciphered the structure of the components critical for transcription (Kornberg 1974; Kornberg and Thomas 1974; Lue and Kornberg 1987; Sayre *et al.* 1992; Chasman *et al.* 1993; Asturias *et al.* 1999; Gnatt *et al.* 2001; Bushnell *et al.* 2002, 2004) while Arthur Horwich's work (awarded the 2011 Lasker award) established the action and mechanism of chaperones (Cheng *et al.* 1989; Ostermann *et al.* 1989; Braig *et al.* 1994; Fenton *et al.* 1994; Rye *et al.* 1997). Given the critical importance of protein synthesis, folding, and aggregation to significant human disorders, such as Huntington's or Alzheimer's disease, the conserved nature of these basic cellular processes has proven that work in budding yeast is significant in another major area of biomedical research. More recently, work involving budding yeast by Jack Szostak, Elizabeth Blackburn, and Carol Greider on eukaryotic telomere structure (Szostak and Blackburn 1982; Murray and Szostak 1983; Blackburn 1984; Dunn *et al.* 1984; Shampay *et al.* 1984; Lundblad and Szostak 1989; Blackburn and Gall 1978; Greider and Blackburn 1985, 1989; Yu *et al.* 1990; McEachern and Blackburn 1995; Kim *et al.* 2001) led to the Lasker Prize in 2006 and a Nobel Prize 2009. This work demonstrated the conservation of basic cellular processes between distant species and brought the power of the tractable yeast system to a broad range of eukaryotic systems. Telomere sequences from *Tetrahymena* were found to protect the ends of chromosomes in budding yeast (Szostak and Blackburn 1982), and mutants were identified that resulted in telomere shortening, demonstrating both a critical function to telomeric sequences and conservation of this mechanism across species. Telomeres are linked to cancer, anemia, and other human conditions, such as aging. In fact, an astounding amount of aging research has been produced from the budding yeast system. In addition to studies of telomere function, budding yeast researchers have related mitochondrial function (oxidative stress and retrograde responses), autophagy, apoptosis, replication stress, and cytoskeletal dynamics to aging. Regulation of cellular differentiation are well studied using yeast, exampled by the pioneering work of Ira Herskowitz describing mating-type switching events in *S. cerevisiae* (awarded the 1985 National Academy of Sciences Award for Excellence in Scientific Reviewing). Of course, we would also be remiss if we did not mention that Herskowitz is credited with the use of the arrow and T bar to indicate positive and negative regulation, respectively (nicely reviewed in Botstein 2004), dramatically influencing how we communicate regulatory circuits. A list of some of the landmark discoveries achieved through the use of the yeast system is presented in Table 4.

## Conclusion

A kitchen companion for centuries, *S. cerevisiae* has seen exponential growth (pun intended) as a laboratory companion over the past half century. The sequencing of strain S288C in 1996 marked the first eukaryotic genome to be sequenced (Goffeau *et al.* 1996). With the genomic sequence and molecular biology tools in hand, the international yeast community embarked on an unprecedented cooperative effort, the yeast deletion project (Winzeler *et al.* 1999). As *S. cerevisiae* has the most advanced selection of genetic tools available for any eukaryotic organism, perhaps even any model organism, it has served as the launch pad for landmark discoveries in gene regulation mechanisms and other cellular processes over the past several decades. The relatively small genome size and ease of culture makes

yeast amenable to high-throughput screening. The data output of high-throughput technologies has led to the development of new fields of computational biology necessary to understand biology at the systems level and work toward building a comprehensive model of the functioning of a eukaryotic cell. The scientific community has access to the compiled genetic and biological information at the excellent online resource, the SGD (http://www.yeastgenome.org/) (Cherry *et al.* 2012). The database includes gene annotations, data from high-throughput screens, and publications with links to other databases and sequence information. In keeping with the tradition of global cooperativity, one of the latest endeavors of the yeast community is the synthetic yeast project, which has the goal of building a completely synthetic strain of *S. cerevisiae* (Dymond *et al.* 2011). Through the rapid development of yeast as a model organism, a delightful discovery has been the surprisingly high level of protein amino acid sequence and functional conservation between yeast and larger eukaryotic species. The conservation of protein amino acid sequence and function, combined with the flexibility of genetic tools, make *S. cerevisiae* a powerful model organism for studying the cellular workings and diseases of larger eukaryotes. In fact, the yeast deletion collection has been used to screen for human diseases (Steinmetz *et al.* 2002). Budding yeast continues to be a most versatile, powerful, and tasty model organism.

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## Glossary of experimental approaches and tools

**BLAST (Basic Local Alignment Search Tool)**: A computational resource whereby a specific nucleotide or amino acid sequence is compared to and aligned with sequences present in one or more of the several available databases using parameters defined by the user. BLAST is a powerful tool that can uncover functional and/or evolutionary relationships between different genes or proteins.

**Chromatin immunoprecipitation (ChIP)**: A powerful biochemical technique that allows investigators to determine the level of occupancy of a protein of interest to a specific location across a genome. In a typical experiment using the yeast model system, cells are grown to logarithmic phase and exposed to the cross-linking agent formaldehyde. Chromatin is then isolated, sheered, and subjected to an immunoprecipitation step using an antibody specific for a protein of interest. The recovered chromatin is then further processed and quantified using quantitative PCR. Assessments of protein occupancy are determined by comparing the levels of precipitation of the regions of interest to those of genomic regions known to not interact with the protein being investigated.

**ChIP-Seq**: A high-throughput version of the ChIP assay in which all of the genomic regions bound by a specific protein of interest are identified using next-generation sequencing technology.

**Complementation tests**: In yeast genetics, a technique used to determine if two independent haploid yeast populations that share a common recessive mutant phenotype do so as a result of mutations in the same gene or in different genes. The technique involves mating the two populations under investigation and testing the resulting diploids for the shared phenotype: presence of the mutant phenotype indicates lack of complementation (*i.e.*, the two populations likely carry mutations in the same gene) and absence of the mutant phenotype indicates complementation (*i.e.*, the two populations likely carry mutations in different genes).

**Functional complementation**: In yeast genetics, a term used to describe the ability of a gene to restore normal function to cells that otherwise display one or more mutant phenotypes due to a genetic mutation. Functional complementation approaches are often used by yeast geneticists as tools to isolate wild-type versions of mutant genes isolated in genetic experiments.

**Genetic crosses**: In yeast genetics, an experimental strategy in which haploid yeast cells of opposite mating types and with different genetic backgrounds are allowed to mate and subsequently stimulated to produce haploid progenies with recombined genomes. Genetic crosses are often used as a way to generate yeast strains with specific combinations of genetic mutations.

**Genetic screens**: In yeast genetics, a technique that allows for independent clonal populations of mutant yeast cells—normally grown as independent colonies or patches on solid medium—to be tested for specific phenotypes to identify mutations in genes of interest.

**Genetic selection**: In yeast genetics, a technique in which yeast cells harboring specific mutations of interest can be identified among large populations of genetically diverse cells based on their ability to grow under conditions that are otherwise detrimental to cells not carrying the mutations.

**Green fluorescent protein (GFP)**: A naturally occurring protein expressed in the jellyfish *Aequorea victoria* with green fluorescence properties. The gene encoding GFP can be fused to genes in other organisms, including yeast, using

molecular biology tools, and the resulting fusion proteins can often be visualized in live cells using fluorescence microscopy. Localization of proteins through the use of GFP fusions has become a powerful tool available to cell biologists, but cannot be universally used as fusions of GFP to certain proteins can lead to their degradation or mislocalization in cells.

**High-copy suppressors**: A term used to describe those genes that, when present in cells at abnormally high copy numbers, can partially or completely mask (*i.e.*, suppress) a mutant phenotype conferred by a mutation in a specific gene. The identity of the high-copy suppressor genes can often provide insights into functional aspects of the mutant gene that they suppress.

**Insertional mutagenesis**: A general term to describe a set of techniques used to cause genetic mutations through the insertion of foreign DNA fragments into the genome of a host cell. Investigators can design the insertions to occur at random locations throughout the genome or to be targeted to specific genomic locations.

**One-step gene replacement**: A term used to describe experiments in which a specific gene is replaced by another gene *in situ* as a result of a single recombination event. This experimental approach, which is highly efficient in the yeast system, relies on the ability of engineered DNA fragments to integrate at specific genomic locations through homologous recombination. In a common application of this technique, a gene under investigation is replaced—and thus knocked-out—by an integrating DNA fragment that carries a nutritional or drug-resistance selectable marker.

**Plasmid recovery**: A general term to describe experimental tools by which one or more plasmids of interest are recovered and purified from cells. Normally, plasmid recovery from yeast cells involves lysing of cells carrying the plasmids and transformation of the released plasmids into *E. coli*, followed by standard plasmid preparation protocols for *E. coli*.

**RNA-Seq**: A high-throughput experimental tool that utilizes next-generation sequencing technology to obtain sequence data representing the entire set of RNA molecules transcribed within a particular cell population.

**Spontaneous suppressor mutations**: Genetic mutations that arise through spontaneous processes, such as through errors during DNA replication, that partially or completely mask (*i.e.*, suppress) a mutant phenotype caused by another mutation. Suppressor mutations are generally categorized as either intragenic (located within the same gene that caused the initial mutant phenotype) or extragenic (located in genomic regions other than the gene that caused the initial mutant phenotype) and can often provide insights into the functional characteristics of the original mutant gene.

**Synthetic Gene Array (SGA) analysis**: A high-throughput experimental platform available to yeast geneticists in which genetic interactions between query mutant strains and libraries of yeast strains carrying deletions of all nonessential genes are screened in a systematic fashion. Types of genetic interactions that can be uncovered using this tool include synthetic-lethal and suppression interactions.

**Synthetic lethal screens**: In yeast genetics, a specific type of genetic screen designed to identify nonlethal mutations that, when combined with a nonlethal mutation in a gene of interest, result in a lethal phenotype. Synthetic-lethal interactions are often indicative of a functional relationship between the wild-type versions of the two genes that partake in the interaction.

**Tandem Affinity Purification (TAP) tag**: An affinity tag whose genetic information can be fused to the coding region of a gene of interest to generate a fusion gene encoding a so-called TAP-tagged protein. TAP-tagged proteins can be biochemically purified from cell lysates using IgG molecules immobilized to a solid support (such as magnetic beads) that specifically interact with the Protein A component of the tag. If desired, a second purification step can be carried out using the calmodulin-binding peptide component of the tag, which binds tightly to calmodulin. Analysis of material that copurifies with TAP-tagged proteins can result in the identification of factors that physically interact with the tagged proteins.

**Tetrad analysis**: An experimental approach used by yeast geneticists to assess the phenotypes—and associated genotypes—of colonies formed by the four haploid products (spores) produced by a single diploid cell through the process of meiosis. This process, which normally involves dissection of tetrads using a microscope equipped with a micromanipulator and subsequent replica-plating of the resulting spore colonies onto a series of solid growth media plates, also allows for the visualization of the segregation of certain traits following meiosis and facilitates assessment of possible genetic interactions between different genetic mutations.

**Transformation**: In yeast genetics, a term used to describe a set of tools used to introduce DNA molecules—commonly in the form of plasmids or linear fragments—into yeast cells. The presence of a newly introduced DNA molecule into a host cell changes, or transforms, its genotype and commonly its phenotype as well.

**Yeast two-hybrid system**: An experimental system used to detect protein–protein interactions in an *in vivo* setting. The yeast two-hybrid system can be used to test if two specific proteins interact with each other or to screen or select for protein–protein interactions between a protein of interest and proteins expressed from a genomic or cDNA library. This system can be used to assess interactions among yeast proteins as well as among proteins from other species.

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