Budding Yeast *Saccharomyces cerevisiae* as a Model Genetic Organism

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Introductory article

Article Contents

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Budding yeast has served as an experimental organism for genetic research for over 50 years. The yeast shares a common cell division cycle and cellular architecture with other eukaryotes, and as a microorganism, it is easily propagated and manipulated in the laboratory. An intense focus on the central dogma, the cell cycle and sexual reproduction unleashed new fields including gene silencing, homologous recombination and differential gene expression, among others. The ease of genetic analysis allowed researchers to study processes to a degree not seen for other model organisms. As was often the case, new techniques were developed in yeast that are now broadly used. Budding yeast was the first eukaryote to be sequenced, which, in turn, led to genome-wide analyses to map gene networks common to all life. Research on yeast has also informed us on the molecular basis of human diseases from birth defects to neurodegenerative disorders.

Introduction

The budding yeast *Saccharomyces cerevisiae* has emerged as a remarkably tractable eukaryotic model system and has played a pivotal role in understanding eukaryotic biology. It is nonpathogenic and easy to grow. Genome engineering is facilitated by its robust homologous recombination capabilities. Both forward and reverse genetic approaches coupled with high-throughput analysis have allowed for the elucidation of the gene networks that underlie eukaryotic cellular function. Analysis of these networks has advanced our understanding of disease. Conservation of yeast genes extends to over 1000 human disease genes (Heinicke *et al*., 2007) including the genetic basis of birth defects, cancer and even neurodegenerative disorders such as Parkinson's, Alzheimer's and Huntington's diseases. The long history of yeast research has led to a wealth of tools and information, utilised not only by yeast researchers but also by the field of genetics as a whole. Collectively, these features contribute to what yeast researchers fondly refer to as the 'awesome power of yeast genetics'! **See also**: **Yeast as a Model for Human Diseases**

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How Yeasts Became Popular Model Systems

Humans have cultivated the budding yeast *S. cerevisiae* since the dawn of agriculture to make beer, bread and wine. As a nonpathogenic, nonmotile microorganism, budding yeast is easily propagated and manipulated in the laboratory and is commonly used in many industries, with hundreds of industrial strains available commercially. This yeast found an early role in metabolic research owing to its association with the fermentation industry (Gallone *et al*., 2016). Early geneticists used yeast and other unicellular fungi to define the underlying genetic control of metabolism. Mutations in metabolic genes soon became useful as selectable markers for genetic analysis, acting as indicators of a strain's genotype. However, yeast meiosis is perhaps the most important reason the budding yeast became such a widely studied model organism for eukaryotes. As all four products of meiosis can be isolated and propagated as haploid organisms, researchers can easily control crosses between strains to interrogate the link between genotype and phenotype at either the single gene level or by genome-wide analysis. **See also**: **Yeasts**; ***Saccharomyces cerevisiae*: Applications**

Studying the mechanisms of homologous recombination related to meiosis and mating-type switching has led to the development of a wide variety of genetic tools to add or replace

DNA (deoxyribonucleic acid) sequences at targeted chromosomal loci. This is a common outcome of yeast research; as scientists have pursued specific biological questions, they have simultaneously developed methodology applicable to a wide range of questions. This is also illustrated by the discovery of the genetic elements important for DNA replication and chromosome segregation, which were co-opted by researchers to develop yeast plasmids and artificial chromosomes that are commonly used today for the analysis of genes and their functions.

In turn, these resources and the massive amount of accumulated data on budding yeast have made it a central component of modern biological research. For example, budding yeast was the first eukaryote to have its entire genome sequenced. Knowledge of the completed genome sequence has led to significant advances for understanding cell and molecular biology (Giaever and Nislow, 2014). Using this information, researchers have generated the yeast gene knockout collection containing ~6000 precise gene disruption mutants and the yeast green fluorescent protein (GFP) fusion collection consisting of over 4000 GFP-tagged full-length open-reading frames (ORFs; Huh *et al*., 2003). Knowing the genome sequence facilitates genome engineering. The Yeast Genome Project (Sc2.0) is an international effort aimed at creating the first synthetic eukaryotic genome (syntheticyeast .org). With this strain in hand, questions related to the genetic mechanisms that control cell fitness and the evolutionary aspects of how transposons evolve and spread throughout a host genome are now tenable. At the time of this writing, strains carrying five synthetic chromosomes representing 30% of the *S. cerevisiae's* genetic material have been created (Maxmen, 2017).

This article focuses on budding yeast as a model genetic organism. Note that other widely studied unicellular fungi, particularly the fission yeast *Schizosaccharomyces pombe* and the orange bread mould *Neurospora crassa*, share many of the traits that make *S. cerevisiae* an attractive model organism and are useful for analysing genetic phenomena that have no parallel in the budding yeast. These differences are noted in the following sections. **See also**: ***Neurospora* Genetics**

Yeast Biology

As a model organism, budding yeast shares many features of cellular biology that are conserved across eukaryotes. Early yeast researchers recognised that studying the mechanistic underpinnings of yeast phenomena could lead to key insights into basic cellular processes. Indeed, many original phage biologists migrated to work on yeast in the 1970s to find whether there were gene regulatory circuits in eukaryotes that acted similarly to those characterised in bacteriophage. Early experiments in yeast focused on understanding the mechanisms underlying metabolism, cell cycle, sexual reproduction and mating-type interconversion. It is not possible to summarise all of yeast biology, so the sections that follow are examples of how research on yeast informed our understanding of fundamental cellular processes with a focus on the contributions of hypothesis-driven research. When appropriate, the first or most definitive paper to report on the topic is cited.

The life cycle

Yeasts grow on moist surfaces that are rich in sugars, amino acids and other soluble nutrients and have the ability to respire to create carbon dioxide and water as primary waste products, or ferment to produce carbon dioxide and alcohol. Humans have taken advantage of this dual biology of *S. cerevisiae* to create leavened bread and fermented drinks such as wine and beer. In the wild, most yeasts grow as diploids when nutrient levels are high, but under starvation conditions, they will enter the sporulation program that is coupled to meiosis to reduce the diploid genome (2N) to produce four haploid spore products (1N). The four spores of a single meiosis are held together as a tetrad, surrounded by a thick wall called an ascus. In this way, spores are more resistant to the environment than are vegetatively dividing cells. When conditions are favourable, haploid spores will germinate and spore clones of the opposite mating type will fuse to generate diploid cells with increased genetic diversity. The switch from the cycling program to the sporulation program requires sensors to monitor nutrient availability, for those sensors to effect changes in gene expression profiles, and for the activation of sporulation-specific genes to produce proteins required for meiosis and spore-wall formation. Studying each of these transitions has led to significant advances in several fields of molecular, cellular and developmental biology as well as evolution.

The cell cycle

Both haploid and diploid cells divide by creating a bud that increases in size as a mother cell progresses through the cell cycle. Following anaphase when the genetic material is equally separated between the mother and the daughter cell, the bud is released to produce a genetically identical daughter cell. As in other eukaryotes, yeast cells age. A mother cell produces a limited number of daughter cells. Lifespan in yeast is under the control of a genetic programme that shares features in common with multicellular animals.

As budding yeast progresses through the cell cycle, its morphology changes (**Figure 1**). In the G_1 phase, cells appear round and unbudded. During S phase, a small bud emerges and continues to grow until cells reach G_2/M phase, when the nucleus is found adjacent to the bud. One notable difference of yeast compared to many other eukaryotes is that it undergoes a closed mitosis, meaning that the nuclear envelope does not break down during the process of chromosome segregation. Leland Hartwell (University of Washington) recognised that budding morphology could serve as a readout of the cell division cycle (CDC). He reasoned that a forward genetic screen to identify mutants that arrested at different developmental stages of budding would also identify genes that regulate cell cycle progression (Hartwell *et al*., 1970). Identification and characterisation of these *CDC* genes have led to a greater understanding of cellular events that lead to cancer cell development, as human homologs of many of the *CDC* genes have been shown to play a role in this process. Dr. Hartwell shared the Noble Prize for his work in elucidating the protein network that controls cell cycle progression with geneticist Sir Paul Nurse, who used fission yeast *S. pombe* and biochemist Sir Richard Timothy Hunt. **See also**: **Cell Cycle**

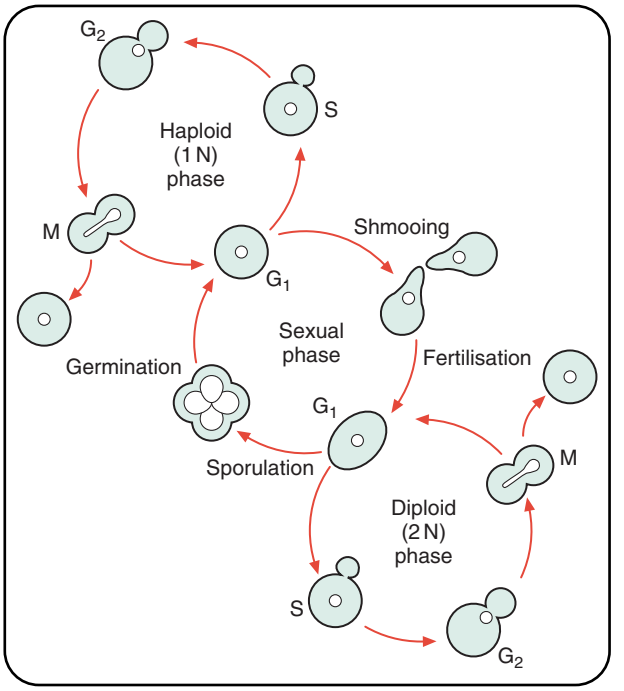

Figure 1 The life cycle of *Saccharomyces cerevisiae*. Cells can be grown vegetatively as haploids or diploid. Sporulation is a cellular pathway that is induced by limited nutrients. The output of sporulation is four haploid spores (tetrad) that is encased in an ascus coat.

Mating types

The budding yeast has two mating types termed *a* and α that are expressed at the mating-type locus *MAT* found on chromosome III. A haploid yeast cell of one mating type produces a pheromone detected by a cell of the opposite mating type, which stimulates fertilisation. The cells first grow projections towards each other in a process called shmooing named after the pear-shaped comic book character created by the British cartoonist Al Capp. The cells then fuse to form a diploid cell, which is *a*/α and cannot mate. The newly formed zygote can divide mitotically to generate more diploid cells (**Figure 1**).

The ability of yeast cells to respond to mating pheromones produced by the opposite mating type involves a complex signal transduction pathway. This pathway begins at the plasma membrane when pheromone binds to and activates a transmembrane pheromone receptor. Once activated, the receptor initiates an intracellular signalling cascade that controls the mating response. Components within this cascade include protein kinases and their regulators, as well as transcription factors essential for the expression of mating-specific genes. The overall architecture of the mating pathway is highly conserved, including proteins within the mitogen-activated protein kinase (MAPK) family of protein kinases. Thus, the mating pathway has been an important paradigm for our understanding how growth factors and other hormones regulate cell growth in mammalian cells.

The mating type of a haploid cell is determined by whether *a* or α information is expressed at the *MAT* locus. The *MAT* α locus encodes two protein products, α1 and α2. The expression of α1 promotes the transcription of α-specific genes, whereas the expression of α2 represses *a*-specific genes. *MAT a1* does not play a role unless the cells are diploid. In diploid cells, both a1 and α2 are expressed and together they repress haploid-specific genes, *a*-specific genes, and α2! These findings were important as they showed that gene circuits could influence developmental programs and that these circuits are similar to those seen in phage (Murray and Gann, 2007).

In the infancy of yeast research, crosses were complicated by homothally, a trait in which a haploid mother cell switched mating type between *a* and α in G1 of alternating mitotic divisions. The outcome of mating-type switching pushed cells to a diploid state due to mating with their siblings. Early researchers wanted to understand how the information at the MAT locus could change. If a cell can switch from *a* to α and back to *a*, it means that the *a* information had not been lost but was somehow silenced. This led to the discovery that cells possess the information for both *a* and α at two transcriptionally silent loci (*HML* and *HMR*) at opposite ends of chromosome III. When a cell switches, DNA encoding the opposite type is copied from the silent locus, and the old mating-type information at *MAT* is degraded. Mating-type switching requires the HO endonuclease that creates a DNA double-strand break (DSB) at the *MAT* locus. This break induces the specific recombination event during G_1 in mother cells. Understanding the mechanism by which a DSB can initiate a nonreciprocal exchange of information at the *MAT* locus led, in part, to understanding the molecular steps of homologous recombination (Haber, 2012).

Most laboratory yeast strains are heterothallic, bearing a mutation in the HO endonuclease. These strains can be traced back to a yeast collected from a rotting fig in Modesto California in 1938 (Greig and Leu, 2009). This allowed researchers to maintain stable haploid cell cultures, unless they are studying the switching process itself. If not for this fig, yeast might not have this claim to fame as a model genetic organism.

The silent mating-type loci (also known as the hidden mating-type loci) offer a simple model of gene silencing. Silent chromatin in budding yeast is modulated through the deacetylation of residues on histones H3 and H4 by the histone deacetylase Sir2 and its associated proteins. Additionally, Sir-dependent silencing occurs near telomeres and the rDNA locus. To study these effects, researchers have designed clever assays to monitor silencing for use in mutant screens to identify silencing information regulators, or *SIR* genes (Rine and Herskowitz, 1987; Gottschling *et al*., 1990). The histone-deacetylase, Sir2, has been shown to be involved in yeast ageing and has been a protein of interest in understanding cellular ageing in higher eukaryotes, including humans. Notably, *S. cerevisiae* lack the histone mark H3K9me2, among others, that is a characteristic feature of heterochromatin in other eukaryotes. In addition, in budding yeast, there is no intracellular machinery for the biogenesis of small RNAs (ribonucleic acids) that can direct silencing at repeated DNA elements, including dicer and argonaute proteins.

The study of mating-type interconversion provided first insight into understanding conserved cellular processes from cell signalling to gene expression, among others. Additionally, the mechanism of mating-type switching has been intensively

investigated as a model for homologous recombination directed by a sequence-specific DSB. This work laid the foundation for the development of gene-editing tools such as CRISPR, which relies on DNA DSB formation and repair. **See also**: **Yeast Mating Type**; **Gene Inactivation Strategies: An Update**

Genome

The budding yeast carries its genome of ∼5800 protein-coding genes in 12 mega base pairs (bp) of DNA on 16 linear chromosomes in the nucleus. The genome is very compact for a eukaryote; the number and size of genes are relatively small and the density of genes along chromosomes is relatively high. Yeast genes have few introns, and intergenic regions are very short. Genetic redundancy in the yeast genome is low, facilitating the analysis of gene function; that is, the relationship between genotype and phenotype can be assessed rapidly by mutating only one gene at a time. The small genome size led to the development of a wide variety of tools for the analysis of gene networks that transcend yeast biology to understanding the molecular basis for many human diseases. In fact, some human genes can be expressed in yeast in place of the endogenous yeast gene, making functional analysis tractable. Human interferon-alpha was among the first human genes to be expressed in yeast (Tuite *et al*., 1982).

Chromosome elements

The linear chromosomes of eukaryotes require at least three DNA elements to be transmitted with high fidelity. Origins of replication are required for initiating DNA synthesis; centromeres (CENs) are necessary for building the kinetochore, the spindle apparatus to segregate chromosomes. Telomeres are needed to maintain the ends of linear DNA molecules during DNA replication. Origins of replication are defined by short discreet sequences, ∼100–200 bp in length, called autonomously replicating sequences (*ARS*). The average distance between ARS sequences is ∼36 kb, although some can be as close as 2 kb (Poloumienko *et al*., 2001). Unlike other eukaryotes, budding yeast has point CENs that are only ∼125 base pairs compared to the regional CENs of fission yeast that span several kilo base pairs, or mega base pairs in human cells. The relatively small size of the *ARS* and *CEN* elements has enabled researchers to create powerful plasmids and artificial chromosomes for a variety of uses, as well as unlocking the mechanisms and genes involved in the replication and segregation of chromosomes. A linear DNA fragment with a *CEN*, an *ARS*, and telomere repeats at the ends can act as a *y*east *a*rtificial *c*hromosome (YAC). These chromosomes were instrumental for DNA sequencing projects as they could contain large contiguous stretches of genomic DNA. The discovery of YACs came about by studying telomerase, the enzyme that adds short repeated sequences to the ends of chromosomes using an RNA template. Elizabeth Blackburn, Carol Greider and Jack Szostak were awarded the Nobel Prize in Physiology and Medicine for their work on telomeres and telomerase. YACs have also been instrumental to identifying genes required for chromosome stability (Shampay *et al*., 1984). Further research to characterise these chromosome elements led to the development of the commonly used chromatin immunoprecipitation assay, known at ChIP, which is commonly used for genome-wide analysis of chromatin-binding proteins in yeast and other organisms. A variation of this method, Hi-C, has been used to assess the organisation of chromosomes in the nucleus (Dekker *et al*., 2002). A model representing the position of each chromosome has been informative in the analysis of nuclear architecture (Duan *et al*., 2012). **See also**: **Yeast Artificial Chromosomes**; **Telomere**

Research Milestones

The utility of yeast has made it an important tool in gene discovery. Genetic and biochemical screens have been instrumental in identifying genes involved in a given process, testing their biological function using purified proteins or cell extracts, and using genome-wide approaches to understanding how networks of genes interact. Information collected by these analyses has been curated in the centralised *Saccharomyces* Genome Database (SGD). This has allowed yeast researchers to contribute to our understanding of many cellular processes that take place in all eukaryotes. Below are just a few biological examples where the use of yeast as a model organism opened up entire new areas of research or the development of new research methods. In several cases, new genetic tools were developed to answer biological question at a level not yet attained in other eukaryotes.

Inducible gene expression

Budding yeast genes are very simple compared to higher eukaryotes. Noncoding regions are sparse, as most yeast genes do not contain introns and intergenic regions are generally free of repetitive DNA sequences. Analysis of proteins that regulate gene expression has given rise to pivotal shifts in understanding. One example of regulated gene expression was given above to describe the regulation of mating type. A second type of gene regulation occurs at the nutrient level. The *GAL* genes have been intensely studied as an inducible expression system that allows yeast to use galactose as a nonfermentable carbon source. Gal4 is a transcription factor that, when bound to a specific sequence, recruits coactivators and the general transcription machinery to promoter regions. When cells are grown in a sugar other than galactose (e.g. glucose), Gal4 is bound to the upstream activating sequence (UAS) but is prevented from recruiting transcription machinery; however, when cells are then shifted to media with galactose as a carbon source, this repression is relieved and *GAL* genes are expressed. Researchers have used this inducible system to control the expression of selected genes fused to the *GAL* promoter region, which has proven powerful for the study of gene function.

Gal4 is an interesting protein as it carries a protein-folding domain that can bind to the UAS DNA sequence and another domain that can recruit the transcriptional machinery necessary to induce transcription (Ma and Ptashne, 1987). This feature of Gal4 is used in the well-known yeast two-hybrid (Y2H) assay to test for protein–protein interactions in cells (Fields and Song, 1989). In this assay, the first protein of interest is fused to the Gal4 DNA-binding domain, called the 'bait', whereas the second protein, called 'prey', is fused to the transcriptional activating domain of Gal4. Expression from a *GAL* promoter is

only possible if the two proteins interact. Expression is typically detected using a reporter gene (e.g. the *lacZ* gene from *E. coli*) that allows for visual colony screening. Variations of this approached have been used to identify protein–DNA interactions by a one-hybrid approach, among others. The ability to survey the expression of nearly every gene expressed under inducible conditions was made possible by the development of microarrays, which became a major tool to analyse gene expression profiles in all eukaryotes (Spellman *et al*., 1998). Microarrays have been largely supplanted by RNA-seq to identify all of the transcripts in a population of cells, yet this also was developed first for yeast (Nagalakshmi *et al*., 2008). **See also**: **Gene Expression in Yeast**; **Two-Hybrid and Related Systems**

Molecular machines

DNA→RNA→protein is the central dogma of molecular biology. This pipeline involves several macromolecular machines that include the replisome, RNA polymerase, the spliceosome, the nuclear pore complex and the ribosome. Yeast genetics and biochemistry has been instrumental to our understanding of how these molecular machineries are built and function. This is highlighted by the 2006 Nobel Prize in Chemistry that was awarded to Roger Kornberg for studies of the molecular basis of eukaryotic transcription, which included the first structure of a eukaryotic RNA polymerase (Cramer *et al*., 2000). Genetic screens using yeast identified protein and RNA components of the spliceosome and how they function together (Vijayraghavan *et al*., 1986; Siliciano *et al*., 1987). These insights have been important, as it has been estimated that ∼94% of human genes are alternatively spliced and ∼50% of disease-causing mutations have an impact on splicing. Other large molecular machines include the ribosome and the nuclear pore complex, both of which have been intensely studied in yeast.

Protein synthesis

Yeast studies have extended our understanding of the mechanism of protein synthesis, in particular translation initiation. This step requires recognition of the AUG start codon by the initiator tRNA bound to the small ribosomal subunit. The small subunit is thought to bind first to the 5′ cap of an mRNA and move towards the start codon by a process called 'ribosomal scanning'. An important test of this model came from the analysis of reporter constructs that possessed highly structured stem-loops within the 5′ untranslated region (5′-UTR) of mRNAs encoding an essential biosynthetic gene. Although these mRNAs could be efficiently translated in wild-type cells, mutants were isolated that could not recognise the start codon in the presence of these stem-loop structures. Remarkably, these mutants turned out to be defective in components that interact with the 5′ cap, including eIF4A, an ATP-dependent RNA helicase that is required for unwinding of structured mRNAs.

An important elaboration on scanning was discovered upon dissection of the mechanism of translational control of the mRNA that encoded Gcn4, a transcription factor expressed under amino acid starvation conditions. It turns out that translation of Gcn4 requires the bypass of several small ORFs that lie upstream of the ORF for Gcn4. This bypass occurs by a process called 'leaky scanning' and allows protein synthesis to be coupled to the nutritional state of the cell. With the advent of genomic approaches for monitoring protein synthesis, in particular ribosome profiling, we have learned that upstream open-reading frames (uORFs) are more prevalent in eukaryotes than was previously appreciated (Brar and Weissman, 2015).

Homologous recombination

DNA DSBs that form from ionising radiation, or by oxidative damage resulting from cellular metabolism, can be lethal to the cell. Two main pathways exist to repair these breaks, the more error-prone nonhomologous end joining and the higher fidelity process of homologous recombination. Homologous recombination is used not only to fix DNA damage but also to switch mating types, segregate homologous chromosomes during meiosis and contribute to genetic variation from one generation to the next. A detailed understanding of homologous recombination at the molecular level has been facilitated by combining genetic analysis with the physical recovery of DNA precursors, intermediates and products of the process by gel electrophoresis. The synthesis of a constellation of observations led to the double-strand break repair (DSBR) model that predicted that crossing over involved an intermediate with two Holliday junctions (Szostak *et al*., 1983). Several predictions of this model have been confirmed and the genes involved at each step of the process have been identified and characterised. For example, the discovery of Spo11, the enzyme that creates DSBs to initiate homologous recombination during meiosis, was discovered by combining an elegant mix of genetics, biochemistry and DNA analysis (Keeney *et al*., 1997). Many of the genes required for homologous recombination discovered in yeast through genetic screens are conserved across all eukaryotes (**see also**: **Meiotic Recombination Pathways**). Defects in these genes can lead to chromosome missegregation and aneuploidy, which is a major cause of birth defects and miscarriages in humans. Homologous recombination is integral to genome engineering in yeast as it can be used to introduce new mutations at a specific chromosomal locus (e.g. two-step allele replacement), replace gene sequences with a selectable marker (e.g. gene knockout) or to recover mutated sequences from a chromosome by gap repair (**Figure 2**). Applications of homologous recombination methods worked out in yeast are now used in gene-editing methods such as CRISPR to create mutations by nonhomologous recombination or introduce site-specific mutations by homologous recombination in response to the introduction of a DSB (see above).

Cellular trafficking

Like all eukaryotes, yeast cells have numerous membrane-bound organelles, including a nucleus, endosymbiotic mitochondria, the peroxisome and the organelles of the secretory pathway. In 2013, Randy Sheckman (University of California, Berkeley) shared the Nobel Prize with James Rothman and Thomas Südhof for his discovery of proteins involved in trafficking vesicles through the secretory pathway, which were discovered based on

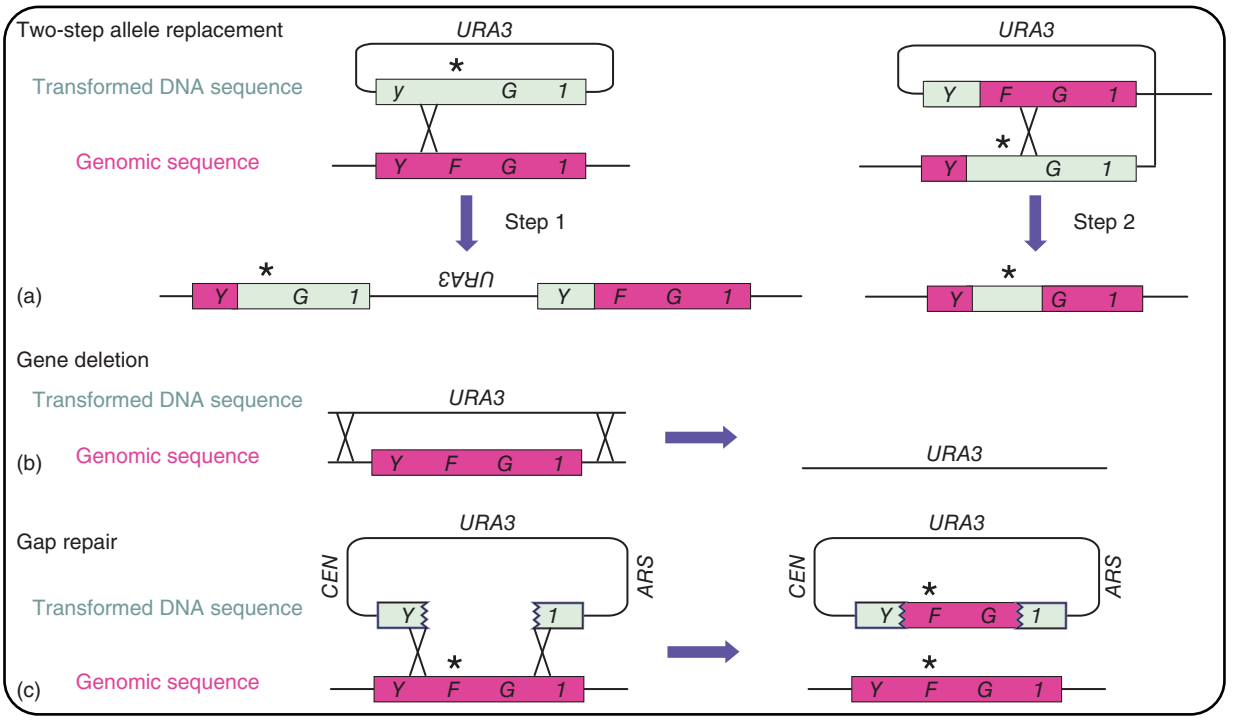

Figure 2 Schematic of genome engineering strategies using homologous recombination. *YFG* is '*Y*our *F*avorite *G*ene' and *URA3* is used as a selectable marker. (a) Two-step allele replacement to replace the wild-type allele of *YFG1* with a mutated allele. For the first step, a strain with the wild-type *YFG1* allele is transformed with a plasmid bearing the mutated *Y*G1* allele and the *URA3* selectable marker. Transformed cells that have integrated the plasmid DNA (deoxyribonucleic acid) sequences into the chromosome by homologous recombination can be selected by growth on media lacking uracil. For the second step, a recombination event between homologous regions will loop out the duplicated sequences and depending on the placement of the recombination event, the mutation will remain in the chromosome. Although this excision event is rare, the relevant strain can be isolated through negative selection against the presence of *URA3* by plating on media containing 5-FOA (5-fluorouracil-6-carboxylic acid monohydrate; 5-fluororotic acid). Cells containing *URA3* will die as the Ura3 enzyme will convert 5-FOA to a product that is toxic to the cell. (b) Allele replacement using a linear DNA fragment with a selectable marker flanked by regions of homology that target integration in place of the wild-type allele. Note that a region of *YFG1* is missing in this fragment. (c) Gene conversion to copy information from the chromosome to a plasmid. Before transformation, a plasmid is cut with restriction enzymes to create a gap in the *YFG1* sequence. This gap is filled with the wild-type sequences in the chromosome by homologous recombination. Note that this plasmid has both *CEN* and *ARS* sequences so that it can be stably propagated in the cell.

genetic screens in the budding yeast (Novick *et al*., 1980). In the secretory pathway, the vacuole, a functional equivalent to lysosomes in metazoans, plays a critical role in recycling cellular material to survive periods of stress, a process termed autophagy. In 2016, Yosinori Ohsumi won the Nobel Prize for his discovery of genes required for autophagy using budding yeast (Tsukada and Ohsumi, 1993). Defects in cellular trafficking in humans are linked to neurodegenerative diseases such as Parkinson's and Huntington's diseases. In fact, the expression of human alpha synuclein has provided insight into the pathology of these diseases (Outeiro and Lindquist, 2003).

The unfolded protein response

The unfolded protein response (UPR) is a pathway that links the accumulation of unfolded or damaged proteins within the endoplasmic reticulum (ER) to an increase in the expression of genes encoding chaperones and other ER-resident proteins. Chaperones boost the protein-folding capacity within the ER. Using genetic approaches in yeast, mutants that were incapable of mounting a UPR response were isolated, which allowed for identification of the components that make up this ER-to-nucleus signalling pathway.

The screen identified three genes. The first encodes Ire1, a multifunctional transmembrane protein localised within the ER membrane that detects the presence of unfolded proteins. This results in activation of the cytosolic portion of Ire1, which possesses both a kinase domain and an unusual RNA nuclease domain. The second gene encodes the transcription factor Hac1, which is required for increased expression of UPR target genes. Interestingly, *HAC1* mRNA is cleaved by Ire1 as part of a noncanonical mRNA-splicing event that occurs independently of the spliceosome. The third encodes the enzyme tRNA ligase, which joins the cleaved exons of Hac1 to form spliced mRNA product that can be efficiently translated to produce active Hac1. Following the identification of this pathway in yeast, it was discovered that the UPR is conserved in higher eukaryotes, where it plays an essential role in the maintenance of cellular homeostasis. As such, the UPR is associated with ageing and the progression of neurodegenerative disease in humans.

TOR: target of rapamycin

Another important example of how experiments in yeast paved the way for our understanding of higher eukaryotes concerns the macrolide antibiotic rapamycin. This drug is produced by a soil bacterium on *Rapa Nui* (Eastern Island), discovered in the mid-1970s (Heitman *et al*., 1993). Soon after, rapamycin was found to be a potent immunosuppressant with important therapeutic value in humans, yet its molecular target was unknown. As rapamycin also inhibits the growth of yeast cells, genetic experiments were used to identify mutants that were resistant to the drug. These studies resulted in the identification of two genes, *TOR1* and *TOR2*, which turned out to encode the protein kinases that are the direct targets of the drug (indeed, TOR stands for target of rapamycin) (Heitman *et al*., 1991). Similar protein kinases are known to be in all eukaryotic cells, where the corresponding protein in mammalian cells is referred to as mTOR. In subsequent years, studies in yeast and other organisms have demonstrated that TOR functions as a central regulator of cell growth in all eukaryotes. This pathway is now the focus for the development of therapies to treat a wide variety of human diseases, including diabetes and cancer.

Prions

Prions are proteins that misfold into a conformation that can induce other proteins to also misfold, which result in protein aggregation and cellular dysfunction. In some cases, these changes are pathogenic and are associated with Mad Cow disease and Creutzfeld-Jacob disease in humans. Some diseases leading to neurodegenerative disorder, including Alzheimer's and Huntington's, are also associated with the formation of protein aggregates. Yeast has emerged as a model system to investigate this phenomenon due to the ability of researchers to express these proteins in yeast and visualise aggregate protein products using tagged fluorescent proteins. With this type of assay in hand, researchers can better understand the normal cellular genes that prevent aggregation. Yeasts have their own prion-like proteins that prevent termination of protein synthesis under stress conditions, thus creating a secondary 'proteome' that could potentially allow the yeast to survive during stress, such as increased temperature (Nizhnikov *et al*., 2014).

The Awesome Power of Yeast Genetics

Budding yeast has played an important part in our understanding of cellular biology of all eukaryotes, and many discoveries that involve yeast have been recognised by the highest scientific prizes, as detailed above. As the biology of yeasts was being discovered, new ways of asking questions became more sophisticated. The constellation of research tools and resources available to yeast researchers is staggering and include a fully sequenced genome, collections of mutants with precise gene knockouts for every gene, the ability to stably propagate freely replicating plasmids, the ability to engineer the genome using homologous recombination, the ability to recover all four meiotic products, the ability to control gene expression, methods to simulate evolution on a lab bench and a vibrant yeast community that openly shares resources and data. The following section outlines approaches one would take to infer a wild-type function of a gene based on its mutant phenotype.

Forward genetic screens

S. cerevisiae is a convenient organism for identifying genes by mutagenesis, having a short generation time and a small genome. Mutations in DNA can result in a loss or gain of gene function. The phenotype conferred by a genetic mutation points to the function of that gene in the cell. Unlike higher eukaryotes, heterothallic yeast strains (those containing a mutation in the HO gene) can be grown in stable haploid or diploid states, thus greatly simplifying the identification and analysis of mutations. In the haploid phase, yeast has one copy of each chromosome, so only one allele is responsible for a Mendelian trait. Thus, the phenotypes of recessive alleles are immediately evident during a mutant hunt, unlike screens for recessive mutations in animals that require two generations. Additionally, as haploids can be propagated and gametes can be specifically chosen after phenotypic evaluation, crosses in the yeast can be precisely controlled.

To find mutations affecting a particular process, a culture of haploid yeast is typically mutagenised with chemicals or radiation and then screened or selected for a mutant phenotype. For example, if a researcher wanted to identify genes affecting uracil biosynthesis, a culture of haploid cells would be mutagenised and spread on an agar-containing petri plate with rich media. Once these mutagenised cells grow into colonies, they are transferred to a dish containing minimal media lacking uracil using the replica plating technique. The yeast on the first plate is pressed on a velvet and then a second plate is use to press that velvet so that the same pattern of colonies are transferred. Colonies with mutations in genes involved in uracil biosynthesis will be unable to grow. By comparing growth on selective and nonselective media, Ura^- colonies are identified and propagated for further analysis. The mutated allele of the *URA3* gene is a popular marker and is denoted in small case letters, *ura3*, indicating that it is recessive to wild type. Nomenclature of budding yeast is presented in **Table 1**.

There are innumerable schemes for screening for mutant phenotypes in the yeast. Conditional alleles are vital for identifying

Table 1 Budding yeast genetic nomenclature for *YFG1* (your favourite gene)

YFG1	Wild-type allele
yfg1-1	Recessive mutant allele
YFG1-2	Dominant mutant allele
*yfg1*Δ	Deletion allele of *YFG1*
yfg1::*URA3*	Insertional disruption of *YFG1* with *URA3*
$Yfg1^+$	Phenotype of *YFG1*
$Yfg1^-$	Phenotype of mutant
Yfg1p	Protein product of *YFG1*
[*YFG1*]	Cytoplasmic genetic factor

Table 2 Complementation test

Known gene		New mutant (*yfgx*)	Diploid phenotype	Interpretation
yfg1-1	*x*	*Wild type*	Wild type	*yfg1-1* is recessive to *YFG1*
Wild type	*x*	*Yfgx*	Wild type	*yfgx* is recessive to *YFGx*
yfg1-1	*x*	*yfgx*	Mutant	*yfg1* and *yfgx* are alleles of the *same* gene
yfg1-1	*x*	*yfgx*	Wild type	*yfg1* and *yfgx* are alleles of *different* genes

and analysing genes essential for survival, so mutant strains can be maintained at the permissive condition (i.e. low temperature), yet die at the nonpermissive conditions (i.e. high temperature). Often it is useful to have more specific secondary screens to find mutants of interest. For example, to isolate *CDC* genes, which are often essential for growth, an initially screened set of temperature-sensitive yeast strains was examined for cell cycle defects (i.e. arrested with different bud morphologies) at the nonpermissive temperature.

Reverse genetics

When the DNA sequence of a known gene is available, yeast is an ideal organism to test for its biological function as homologous recombination can be used to delete the gene. As already mentioned, the budding yeast has a high rate of homologous recombination to repair DNA DSBs. This feature also allows researchers to precisely engineer yeast strains by targeted integrative transformation. Free DNA ends are not well tolerated in yeast, except for the repeated sequences added to the ends of chromosomes by telomerase. Other free ends undergo homologous recombination. If a DNA fragment generated in the lab is transformed into yeast, the two free ends will invade and recombine with endogenous sequence homology and replace the endogenous gene at its chromosomal site (**Figure 2**). Although the ends of the DNA fragment are homologous to the target allele, a selectable marker, usually the wild-type allele of a metabolic gene (such as *URA3*), is nearly always included on the engineered extrachromosomal elements. This product, typically generated using PCR (polymerase chain reaction), is then transformed into an appropriate auxotrophic strain of yeast (such as *ura3*Δ), so that transformed yeast can be selected on media deficient for that metabolite. Nonyeast genes that confer resistance to drugs that would otherwise cause cell death (e.g. KanMX) are also commonly used. Reverse genetics has been greatly simplified by the yeast deletion collection. Now researchers can take the strain from the freezer and immediately begin to test for a phenotype. The entire collection can also be interrogated as in a forward genetic screen. Instead of random mutagenesis, the yeast deletion collection can serve as the pool of 'mutagenised yeast'.

Dominance and complementation tests

When a mutant of one mating type is crossed to wild-type yeast of the opposite mating type, the phenotype of the resulting diploid indicates whether the mutant allele is dominant or recessive. For example, if a wild-type *MATa URA3* haploid is crossed to a *MAT*α *ura3* mutant haploid, unable to grow in the absence of uracil (Ura$^-$), the resulting diploid would have a phenotype of Ura$^+$, able to grow in media lacking uracil. In this case, the mutant *ura3* allele is recessive to the wild-type *URA3* allele. Note the use of lower case and upper case letters to denote recessive and dominant phenotypes (**Table 2**).

When two mutant haploids have the same phenotype, for example, Ura$^-$, the mutations may or may not be in the same gene. If both mutant alleles are shown to be recessive, the phenotype of the diploid indicates whether or not the two alleles reside at the same locus. This is called a complementation test. If the diploid is Ura$^+$, the mutations complement and probably reside at different loci. If the diploid is Ura$^-$, the mutations do not complement and most likely reside in the same gene. The complementation test must be interpreted with caution as mutations in different parts of a single multifunctional gene may complement, whereas mutations in different genes with physically interacting products may fail to complement as was seen for mutations in the *TUB2* gene, encoding tubulin (Stearns and Botstein, 1988). Although these scenarios are rare, they are testable by segregation analysis, discussed below. With large numbers of mutants in hand, the complementation test allows a researcher to rapidly determine how many genes affect a particular phenotype. Further phenotypic analysis of double mutants, called epistasis analysis, orders genes into pathways that control a specific process (see below).

Tetrad analysis

Before the yeast genome was sequenced, one major advantage of tetrad analysis was to map the order of genes onto chromosomes. Although tetrad analysis is an important tool to study the chromosome events of meiosis, it is also frequently used to generate strains of different genotypes. The analysis of strains containing more than one mutation can reveal genetic interactions that can inform gene function (see below). As all four meiotic products of yeast are viable, tetrad analysis is powerful and straightforward. The tetrad of spores is encased in an ascus coat, which needs to be broken down to release the spores. This is generally done enzymatically. Originally, this was done using the snail gut enzyme called glusulase, yet today most researchers use a purified protein called zymolyase. Following treatment to release the spores, a thin needle is used to pick up each spore individually and place them into rows on solid media using a joystick micromanipulator (**Figure 3**). The haploid colonies (or spore clones) that arise are replica-plated to different types of media to evaluate the phenotype (and thus genotype) of individual spore isolates for segregating genetic markers. When a mutant with a single underlying chromosomal mutation is crossed to wild type, the ratio of mutant to wild-type phenotype of spore clones will be 2:2. This

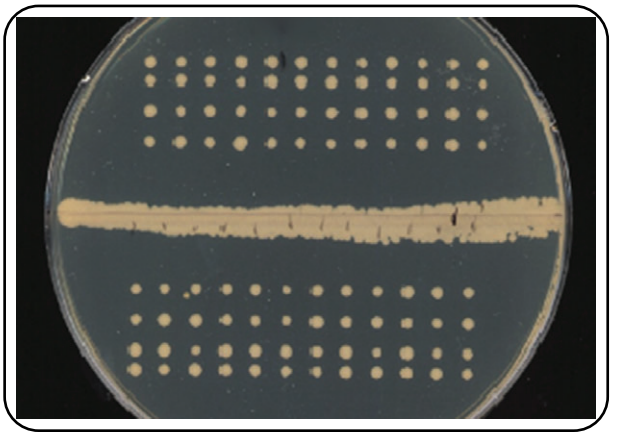

Figure 3 Spore clones of dissected tetrads from a wild-type strain on nutrient-rich media containing yeast extract, peptone and dextrose (YPD) and grown for 2 days at 30 °C. Each colony arose from a single spore of a tetrad. The middle line of cells represents the pool of cells that was used to find tetrads for microdissection.

simple common test reveals whether a particular mutant phenotype is caused by a single locus.

When two markers (e.g. mutations or selectable marker) are followed in a cross, both will show 2:2 segregation, but when they are evaluated together, three classes of tetrad can result. These are the parental ditype (PD), nonparental ditype (NPD) and tetratype (TT). For the PD class, the two markers segregate away from one another at meiosis I. Due to Mendel's law of independent assortment, the NPD class occurs when the two mutations segregate together. If these genes are also unlinked from their CEN, the PD:NPD:TT ratio would be 1:1:4. This is only in theory as the frequency of TTs would be lower for CEN-linked genes. When the two mutations are linked on the same chromosome, the frequency of PD will be greater than TTs. NPDs will be rare as only a double crossover involving all four chromatids can generate this class. The PD:NPD:TT rations for these different scenarios is illustrated in **Figure 4** and **Table 3**.

Cloning by complementation

Yeast plasmids are extremely useful for cloning genes. In order to clone the gene affected by a recessive mutation, a wild-type genomic library (a mixture of plasmid vectors bearing heterogeneous genomic inserts) is transformed into the mutant yeast to screen for a plasmid that complements the mutation (i.e. alleviates the mutant phenotype). Plasmids recovered from rescued yeast may contain the wild-type yeast gene. DNA sequencing will determine the identity of the gene(s) on the plasmid. The cloned sequence may not be the gene originally mutated, however. For example, it could be a gene that suppresses the mutant phenotype when in high copy. The next step then is to knock out that gene at its endogenous location in the genome and test whether it confers the original mutant phenotype (**Figure 2**). By crossing these new strains to the original mutant and dissecting tetrads, segregation analysis determines whether the original mutant and the cloned sequence are allelic.

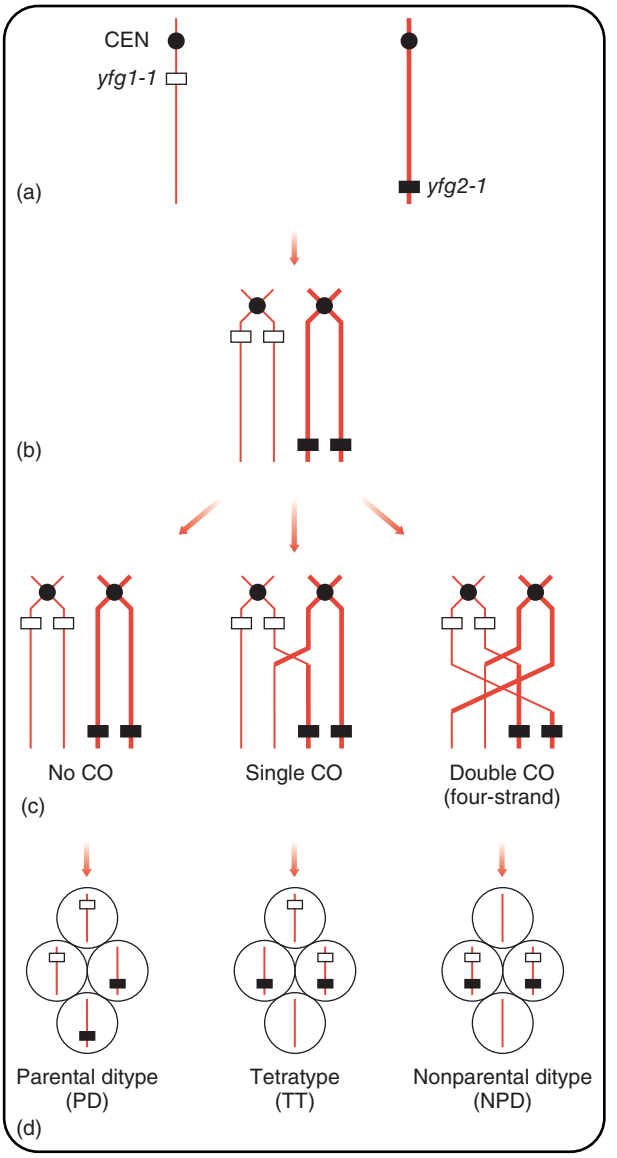

Figure 4 Meiotic segregation in the budding yeast. (a) Cross between two mutants, *yfg1* × *yfg2*. The white and black boxes represent the mutant alleles of *yfg1* and *yfg2*, respectively. The black circle represents the centromere. (b) Schematic of chromosomes after DNA replication and the pairing of homologous chromosomes. (c) Schematic of three possible chromosome configurations after recombination and synapsis. CO stands for crossover. (d) Tetrad classes resulting from a given crossover event are represented with the genotype indicated in the spore. Not shown: two-strand double crossovers result in PD (parental ditype); three-strand double crossovers result in TT (tetratype).

Table 3 Results of a two-factor genetic cross

All PD	Allelic markers
4 T = 1 PD = 1 NPD	Unlinked markers
PD > T ≫ NPD	Linked markers
PD = NPD ≫ T	Unlinked markers, linked to CEN

Table 4 Examples of suppression and synthetic lethality in a tetratype

Genotype	Genotype	No interaction	Extragenic suppression	Synthetic lethality
YFG1	*YFG2*	Viable	Viable	Viable
YFG1	*yfg2-1*	Viable/mutant	Viable/mutant	Viable/mutant
yfg1-1	*YFG2*	Viable/mutant	Viable/mutant	Viable/mutant
yfg1-1	*yfg2-1*	Viable/additive	Viable	lethal

Epistasis: suppression and synthetic lethality

Yeast has been an important system for gene discovery as determining the relationship of phenotype and genotype is straightforward when considering mutations in unrelated genes. The ability to rapidly generate and characterise mutations has also enabled the recovery of second-site mutations that can suppress (become more like wild type) or enhance (increase the severity) a given mutant phenotype. A comparison of single mutant and double mutant combinations can provide important information as to whether genes act in the same or different pathways (**Table 4**). For example, if two genes act in the same pathway, then the phenotype of the double mutant will resemble the parent with the more severe phenotype. However, if the genes have partially overlapping functions, but normally act in different pathways, the double mutant may give a more severe phenotype than either single mutant. When the double mutant is inviable, the relationship is called synthetic lethality, but other types of synthetic phenotypes exist. Thus, ease in creating double mutants makes yeast an ideal system to understand how genes function together. Synthetic genetic array (SGA) analysis in yeast employed the yeast deletion collection to explore the pairwise relationships genome wide, creating ~23 million mutant combinations and a genetic wiring map of a eukaryotic the cell (Costanzo *et al*., 2016). This work was made possible by the following features of yeast: (1) sequenced genome; (2) the use of selective markers; (3) the ability to efficiently make mutations by homologous recombination; (4) automated tetrad analysis and (5) an ability to grow strains in both a haploid and diploid state. A central repository for storing and analysing quantitative genetic interaction data produced by SGA can be found at TheCellMap.org.

There are many ways that double mutants could be less severe or more severe. These mechanisms can be studied in more detail by combining multiple experimental approaches (e.g. to test whether two purified gene products interact physically in a test tube, if a protein can catalyse a enzymatic activity alone or in a complex, test whether two gene products localise to the same organelle, etc.). In this way, extensive gene/protein networks established in yeast create a framework for understanding cellular functions across all domains of life.

Conclusion

Budding yeast continues to be at the forefront of model genetic organisms to study life-supporting processes. Hypothesis-driven research to understand aspects of the cell cycle and mating-type interconversion opened up entirely new fields of study. To answer these and other biological questions, researchers have developed new approaches that also have been broadly applicable to study other eukaryotes. One challenge of the future will be to continue to store, annotate and cross-reference an increasing amount of data. Multidisciplinary collaborations will become increasingly important to understand a process at structural, molecular, cellular, biochemical, computational and phylogenetic levels. Developing new methods to assay phenotypes will increasingly draw on other fields such as physics, mathematics and data science. It is also important to maintain public databases such as the SGD (www.yeastgenome.org) and BioGrid (thebiogrid.org) to store, annotate and cross-reference increasing amounts of data and for researchers to have access to algorithms to generate testable hypotheses. It is certainly an exciting time to be a yeast researcher!

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Glossary

ARS Autonomous replication sequence; the site of initiation of DNA synthesis in eukaryotic organisms.

Centromere (CEN) It is usually defined genetically as the region of a chromosome that always segregates from its homolog during meiosis. Also refers to the region of the chromosome to which spindle fibers attach during cell division, mitotic or meiotic.

Diploid An organism having TWO sets of chromosomes, also called 2N.

Forward genetics A strategy to identify genes involved in a particular process by isolating and characterising mutants defective in that process. The flow of information starts with phenotype to determine genotype. See also 'Reverse genetics'.

Haploid An organism having only one set of chromosomes (1N), with only one copy of each locus in the genome.

Knockout A mutation that removes all coding sequence of a gene. Usually refers to an engineered mutation introduced by transformation and homologous recombination.

Marker An allele whose phenotype can be easily identified and can be used to follow a specific chromosome or a specific organism. Selectable markers are often used to identify transformed yeast (e.g. the yeast knockout collection).

A screenable marker would be an allele detectable by an assayable activity of the gene product (e.g. a fluorescent tag).

Mutant (noun) A strain that has a phenotype differing from that of the wild-type or native strain. (adj.) Used to denote mutated form of gene, allele, protein, base pair, and so on.

Mutation A change in the DNA sequence that is typically induced by the formation and repair of DNA damage or nucleotide misincorporation during DNA synthesis.

Plasmid An extra-chromosomal genetic element generally capable of autonomous replication and transmission to progeny cells. Yeast plasmids are grouped into several categories based on replication ability or function (as defined by the molecular biologist, not the cell).

Promoter It refers to the noncoding regions of DNA that function in the regulation of gene expression.

Recombination The rearrangement of genetic material typically associated with the repair of DNA double-strand breaks.

Reporter gene A gene encoding a conveniently assayable protein that can be used to monitor promoter activity or other genetic events.

Reverse genetics A strategy to assess the functions of known genes by creating mutations or knockouts and assessing the corresponding phenotype(s). The flow of information starts with known genotype to an assayable phenotype. See also 'Forward genetics'.

Shuttle vector A piece of DNA capable of self-replication in two completely different organisms, such as *E. coli* and *Saccharomyces*; allows replication of the vector in both yeasts.

Tetrad Four meiotic products, or spores, that remain associated after meiosis and allow for analysis of chromosome segregation patterns.

YAC Yeast artificial chromosome. It contains sequences necessary for the segregation of linear DNA species in yeast, including a centromere, telomeres and marker genes.

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