CHAPTER 13
Yeast

INTRODUCTION

Saccharomyces cerevisiae (baker's yeast) and *Schizosaccharomyces pombe* (fission yeast) are often considered to be model eukaryotic organisms, in a manner analogous to *Escherichia coli* as a model prokaryotic organism. Both yeasts have been extensively characterized and their genomes completely sequenced. They are as easy to grow as other microorganisms, and they have a haploid nuclear DNA content only 3.5 times that of *E. coli*. However, despite the small genomes sizes, these yeasts display most of the features of higher eukaryotes. The fact that many cellular processes are conserved among different eukaryotic species-combined with the powerful genetic and molecular tools that are available- has made these yeasts important experimental organisms for a variety of basic problems in eukaryotic molecular biology.

Primarily for historical reasons, most studies on yeast have involved *Saccharomyces cerevisiae* (hereafter termed yeast). Culturing yeast is simple, economical, and rapid, characterized by a doubling time of ~90 min on rich medium. In addition, yeast has been well adapted to both aerobic and anaerobic large-scale culture. Cells divide mitotically by forming a bud, which pinches off to form a daughter cell. The progression through the cell cycle can be monitored by the size of the bud; this has been used to isolate a large collection of mutants (called *cdc* mutants) that are blocked at various stages of the cell cycle. Since yeast can be grown on a completely defined medium (see *UNIT 13.1*), many nutritional auxotrophs have been isolated. This has not only permitted the analysis of complex metabolic pathways but has also provided a large number of mutations useful for genetic analysis. Mutations can be generated by classical UV or EMS mutagenesis (*UNIT 13.3B*) or by transposon mutatgenesis (*UNIT 13.3*), which allows one to identify the mutation via the inserted transposon.

Yeast can exist stably in either haploid or diploid states. A haploid cell can be either of two mating types, called **a** and α. Diploid **a**/α cells—formed by fusion of an α cell and an **a** cell (*UNIT 13.2*)—can grow mitotically indefinitely, but under conditions of carbon and nitrogen starvation will undergo meiosis. The meiotic products, called *spores*, are contained in a structure called an *ascus*. After gentle enzymatic digestion of the thick cell wall of the ascus, the haploid spore products can be individually isolated and analyzed (*UNIT 13.2*). This ability to recover all four products of meiosis has allowed detailed genetic studies of recombination and gene conversion that are not possible in most other eukaryotic organisms. The existence of stable haploid and diploid states also facilitates classical mutational analysis, such as complementation tests and identification of both dominant and recessive mutations.

The haploid yeast cell has a genome size of about 15 megabases and contains 16 linear chromosomes, ranging in size from 200 to 2200 kb. Thus, the largest yeast chromosome is still 100 times smaller than the average mammalian chromosome. This small chromosome size, combined with the advent of techniques for cloning yeast genes as well as manipulating yeast chromosomes, has allowed detailed studies of chromosome structure. Three types of structural elements required for yeast chromosome function have been identified and cloned: origins of replication (*ARS elements*), centromeres (*CEN elements*), and *telomeres*. The cloning of these elements has led to the construction of

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artificial chromosomes that can be used to study various aspects of chromosome behavior, such as how chromosomes pair and segregate from each other during mitosis and meiosis. In addition, systems using artificial chromosomes have been designed that allow cloning of larger contiguous segments of DNA (up to 400 kb) than are obtainable in other cloning systems. These structural elements, as well as cloned selectable yeast genes, have permitted the construction of yeast/*E. coli* shuttle vectors that can be maintained in yeast as well as in *E. coli* (*UNITS 13.4 & 13.6*).

Procedures for high-efficiency transformation of yeast (*UNIT 13.7*) have been available for nearly two decades, allowing cloning of genes by genetic complementation (*UNITS 13.8 & 13.9*). Because yeast has a highly efficient recombination system, DNAs with alterations in cloned genes can be reintroduced into the chromosome at the corresponding homologous sites (*UNIT 13.10*). This has permitted the rapid identification of the phenotypic consequences of a mutation in any cloned gene, a technique generally unavailable in higher eukaryotes. In addition, homologous recombination permits a wide variety of genetic techniques that have greatly facilitated the analysis of biological processes.

Despite its small genome size, yeast is a characteristic eukaryote, containing all the major membrane-bound subcellular organelles found in higher eukaryotes, as well as a cytoskeleton. Yeast DNA is found within a nucleus and nucleosome organization of chromosomal DNA is similar to that of higher eukaryotes, although no histone H1 is present. Three different RNA polymerases transcribe yeast DNA, and yeast mRNAs (transcribed by polymerase II) show characteristic modifications of eukaryotic mRNAs [such as a 5′ methyl-G cap and a 3′ poly(A) tail], although only a few *S. cerevisiae* genes contain introns. Transcriptional regulation has been extensively studied and at least one yeast transcriptional activator has been shown to function in higher eukaryotes as well. High-molecular-weight yeast DNA and RNA can be prepared fairly quickly (*UNITS 13.11 & 13.12*). Another characteristic of eukaryotes is the proteolytic processing of precursor proteins to yield functional products, which is often coupled to secretion. Yeast has several well-studied examples of secreted proteins and pheromones, and the large number of genes that have been identified as involved in protease processing and secretion suggests a highly complex pathway. Yeast protein extracts can be prepared using three different protocols (*UNIT 13.13*); the best choice will depend on the particular application. The ease and power of genetic manipulation in yeast facilitate the use of this organism to detect novel interacting proteins using the two-hybrid system or interaction trap (*UNIT 20.1*).

Although *Saccharomyces cerevisiae* is the most commonly studied yeast, *S. pombe* is also an important experimental organism (*UNIT 13.14*). Although both yeasts are unicellular microorganisms that grow in similar medium, they are evolutionarily quite distant. It has become increasingly clear that, in terms of molecular mechanisms, *S. pombe* is more similar to higher eukaryotic organisms than *S. cerevisiae*. Experimental manipulations in S. pombe are broadly similar to those in *S. cerevisiae*, although the technical details often differ. The chapter includes units on *S. pombe* relating to strain maintenance and media (*UNIT 13.15*), growth and genetic manipulation (*UNIT 13.16*), and introduction of DNA into cells (*UNIT 13.17*).

This chapter is written for the molecular biologist who has not previously worked with yeast. The glossary below introduces the terms of yeast molecular biology.

aerobic growth growth in the presence of oxygen, utilizing the Krebs cycle.

α and a factor mating type–specific polypeptides secreted by either α or **a** haploid cells, respectively, which interact with haploid cells of the opposite mating type to stimulate mating.

anaerobic growth growth in the absence of oxygen, utilizing fermentation (via glycolysis).

ARS elements DNA sequences present throughout the yeast genome that confer autonomous replication on plasmids in yeast; most of these sequences also function as chromosomal origins of replication as well.

ascus thick-walled sac containing the four haploid products, called spores, resulting from meiosis.

cdc mutants strains of yeast that exhibit stage-specific blocks of the cell cycle; these mutations define genes important in DNA replication, meiosis, and sporulation.

CEN element DNA sequences present at the centromere that ensure proper segregation of chromosomes during mitosis and meiosis, presumably by promoting interaction with the mitotic spindle.

***cir*$^+$** strains of yeast that contain the naturally occurring 2μm plasmid.

cir^0 yeast strains that have lost this endogenous 2μm plasmid.

δ element ~330-bp sequence, present as direct repeats at the ends of the transposable element Ty1, and also found dispersed throughout the genome.

gene disruption a mutation constructed in vitro in a cloned gene which, upon reintroduction into the genome at the homologous chromosomal site, results in inactivation of the gene function.

glusulase a digestive enzyme isolated from snails that breaks down thick cell walls of either an ascus to allow isolation of spore products, or a yeast cell to produce spheroplasts.

heterothallic common laboratory strains of yeast which—due to a mutation in the *HO* gene—stably maintain a given allele at the *MAT* locus.

homothallic strains of yeast (typically found in the wild) that, in a haploid state, rapidly interconvert the *MAT* locus, resulting in rapid switching between the α and **a** mating types; cultures of such strains rapidly diploidize.

killer strains strains of yeast that harbor a double-stranded RNA virus; such strains kill sensitive yeast strains via secretion of a protein toxin, to which killer strains are immune.

MAT the mating-type locus which is expressed and therefore determines the mating type of a haploid cell; this locus has two alleles—the *MAT***a** allele confers the **a** mating type, while *MAT*α specifies the α mating type.

meiosis the process by which the number of chromosomes present in a diploid cell is halved to yield haploid products.

mitosis vegetative cell division (of either haploid or diploid cells) in which the chromosome number stays the same.

petites mutants of yeast (either nuclear or mitochondrial) with impaired mitochondria function; they grow as small colonies on fermentable carbon sources and are unable to grow on nonfermentable carbon sources.

schmoo a distinctive shape of a haploid cell (pear-shaped), induced by exposure to mating pheromone.

sporulation the end product of meiosis, induced by carbon and nitrogen starvation of a diploid cell, which results in four haploid progeny contained as spores within an ascus; this complicated developmental process requires over 200 genes.

telomeres DNA sequences found at the end of linear chromosomes that are essential for chromosome stability and complete replication; in *S. cerevisiae*, telomeres consist of tandem repeats of the sequence $5'dG_{1\text{-}3}dT3'$.

Ty1 elements the primary transposable element found in yeast, which functions as a retrotransposon (via reverse transcription of its RNA and subsequent reinsertion into the genome).

UAS element upstream activating sequences in yeast promoters, to which regulatory proteins bind in order to enhance the rate of transcription.

zygote a morphologically distinct cellular structure formed by the fusion of two haploid cells of opposite mating type, which results in formation of a diploid.

Zymolyase β-glucanase, isolated from *Arthrobacter luteus*, that hydrolyzes the yeast cell wall and is used to prepare spheroplasts for a variety of purposes.

KEY REFERENCES

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