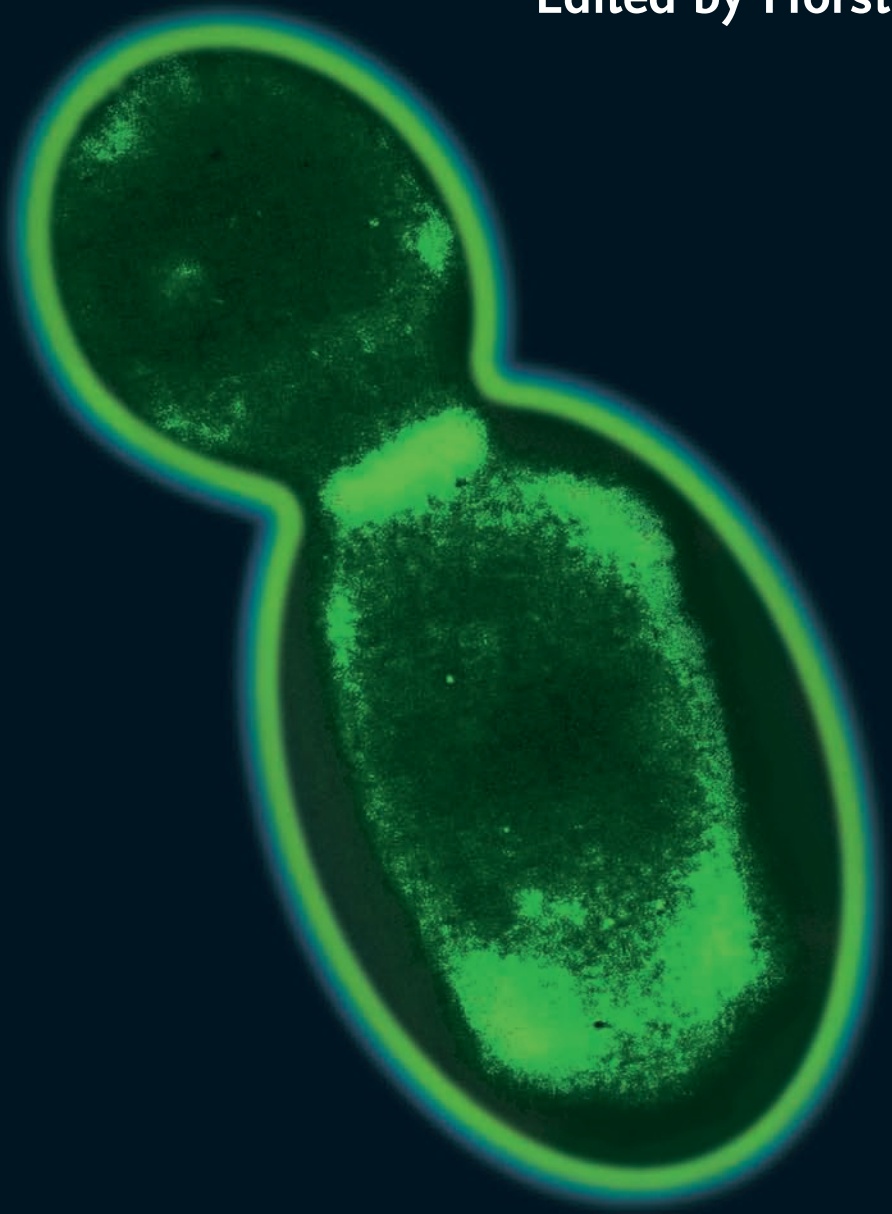
Yeast

Molecular and Cell Biology

Second, Completely Revised, and Greatly Enlarged Edition

Edited by Horst Feldmann



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With contributions from Paola Branduardi, Bernard Dujon, Claude Gaillardin, and Danilo Porro

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Budding yeast marked with GFP.

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Preface

For the Second Edition

Until some 20 years back, there was no need to write a book on yeast molecular and cellular biology: the field was covered by "standard monographs" such as Broach, J.N., Pringle, J.R., and Jones, E.W. (eds) (1991) *The Molecular and Cellular Biology of the Yeast* Saccharomyces, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY., and Guthrie, C. and Fink, G. (eds) (1991) *Guide to Yeast Genetics and Molecular Biology*, Academic Press, San Diego, CA. Unfortunately, these editions were not updated, so that any novel information after the Yeast Genome Sequencing Project had succeeded in 1996 was scarcely available in a comprehensive form.

When I discussed this drawback with my colleagues during the first years of the "postgenome" era, it was André Goffeau who suggested to me that we should at minimum publish a paper documenting the outstanding contributions that had involved *Saccharomyces cerevisiae* as a model system for eukaryotic molecular and cell biology for over half a century. Finally, however, my engagement in this subject ended in preparing a small volume describing all those achievements.

I had started working with yeast in 1962, so that I still retain reminiscences of things happening in the past 50 years. Over the years, I had kept a collection of papers documenting the achievements in various fields of yeast research. I also gained a lot of information from the weekly seminars that were arranged in the departments where I worked, and from lectures and courses that I had a chance to present. For teaching purposes, I kept a huge collection of tables and figures that I personally had designed. I gratefully remember the many fruitful discussions with my colleagues from all over the world – at congresses or privately – that helped broaden my background.

Unfortunately, the brochure, entitled "Contribution of Yeast to Molecular Biology: A Historical Review," did not raise the interest of a publisher, by using the argument " . . . history does not sell . . . " Nonetheless, they became interested in the subject itself after I had converted it into a "modern" textbook (which still might retain notes on historical background), because such an item was absolutely missing on the market. Thus, the first edition of *Yeast: Molecular and Cell Biology* appeared in November 2009.

The necessity to update and publicize information on yeast was recently raised in an article ("Yeast: an experimental organism for 21st century biology") by our American colleagues (Botstein and Fink, 2011). In the November 2011 issue of *Genetics*, the Genetics Society of America launched its *YeastBook* series – a comprehensive compendium of reviews that presents the current state of knowledge of the molecular biology, cellular biology, and genetics of *S. cerevisiae*.

This second edition of *Yeast: Molecular and Cell Biology* was started more than a year ago, and is aimed at presenting all aspects of modern yeast molecular and cellular biology, starting from the "early" discoveries and trying to cover the most recent developments in all relevant topics. The reader will find included chapters that reach out to yeast species other than *S. cerevisiae*, which have turned out (i) as interesting objects for large-scale genome comparisons, (ii) as ideal organisms to follow genomic evolution, and (iii) as appropriate "cell factories" in biotechnology. I think this will fulfill all of the requirements of a textbook for students and researchers interested in yeast biology.

I have tried to document the developments by including more than 3000 references. Whenever possible, these references are selected such that the reader can follow achievements made over the past decades to the present (in addition, a number of individual chapters include a list of references for recommended "Further reading"). Undoubtedly, this collection will not completely mirror the engagement of the numerous yeast laboratories. Wherever possible, I have cited original papers, but in many cases I have had to rely on review articles contributed during these years by competent researchers. Therefore, I apologize to all colleagues who might be disappointed that their original work has not been quoted adequately.

Foremost, I again wish to thank André Goffeau and Jean-Luc Souciet, who supported me in preparing this book. I am indebted to Danilo Porro and Paola Branduardi (Univerity of Milan Biococca), Claude Gaillardin (INRA, Thiverval-Grignon), and Bernard Dujon (Institut Pasteur and Institut Pasteur and University P. & M. Curie, Paris) for their excellent contributions of Chapters 14, 15 and 16, respectively. Not to forget the nice contacts with so many colleagues I found during the Yeast Genome Sequencing Project and the Génolevures Project; I am grateful for their suggestions and encouragement.

With great pleasure, I wish to acknowledge the care of the team of Wiley-Blackwell publishers at Weinheim (Germany) in editing and manufacturing this book: Dr Gregor Cicchetti (Senior Commissioning Editor, Life Sciences), who kindly invited me to consider a second edition with a considerable extension of the contents, and Dr Andreas Sendtko (Senior Project Editor) and his colleagues who took over production. Many thanks for their excellent and accurate handling of my manuscript and the pictures, so that I had little trouble with corrections.

Finally, but most importantly, I wholeheartedly thank my wife Hildegard for her patience and encouragement, who for many years found me toiling over my computer at home.

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Bergkirchen
June 2012

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1 Introduction

1.1 Historical Aspects

In everyday language, yeast is synonymous for *Saccharomyces cerevisiae* – a name given to a yeast strain discovered in malt in 1837 (Meyen) – in connection with making beer. This notion immediately calls to mind that yeast probably is the oldest domesticated organism – it was used for beer brewing already in Sumeria and Babylonia around 6000 BC. In parallel, *S. cerevisiae* strains were employed in wine production in Georgia and for dough leavening in old Egypt. In Egypt, beer was a common refreshment, and gifts of beer were awarded to civil servants and workers for extraordinary services. The scientific name "*Saccharomyces*" is derived from a word meaning "sugar fungus" in Greek, while the root for *cerevisiae* stems from Ceres, the Roman God of the crops.

The French word for yeast, *levure*, goes back to Latin *levare*, and so is *leaven*, simultaneously used for dough and yeast as an organism able to anaerobically release carbon dioxide during the baking process. The English word *yeast*, like Dutch *guist*, or even the German *Hefe*, is derived from a west-Germanic expression, *haf-jon*, meaning the potential to leaven. The provenance of the words used for beer in western European languages (French "bière," German "Bier," and Italian "birra") is not known, but in Roman languages, the expressions used for beer are directly related to the organism (*cerevisiae*), most obvious in the Spanish "cerveza" or in the Portuguese "cerveja." The Greek *zymi* (ζυμι) is used simultaneously for yeast and dough, and occurs as a root in words related to beer or fermentation. Thus, the modern expression "enzymes" (*en zymi* = in yeast), originally coined by Kühne in 1877, designates the compounds derived from yeast that are able to ferment sugar.

We owe the description of the microscopic appearance of yeasts in 1680 to Antoni van Leeuwenhoek in Leiden, who also observed bacteria and other small organisms for the first time. The observation that yeast budding is associated with alcoholic fermentation dates back to Cagnaird-Latour in 1835. In his work carried out during his tenure at Strasbourg University, Louis Pasteur correlated fermentation with yeast metabolism (1857). Pasteur's famous "*Études sur la bière*" appeared in 1876. Sometime later, two technical applications were based on this notion. In the late 1880s, E. Buchner and H. Buchner used cell-free fermentation to produce alcohol and carbon dioxide, and in 1915, Karl Neuberg used "steered" yeast fermentations to produce glycerol (unfortunately as a convenient source to convert it into trinitroglycerol). The knowledge of yeast physiology, sexuality, and phylogeny was later reviewed in a book by A. Guilliermond (Guilliermond, 1920).

In the 1950s, when yeast research entered a novel era of biochemistry, researchers became aware that many useful compounds could be isolated from yeast cells. Among the first companies to produce biochemicals from yeast (nonengineered at that time and obtained from a local Bavarian brewery) for the biochemical and clinical laboratory was Boehringer Mannheim GmbH in Tutzing (Germany). In a "semi"-industrial procedure, a variety of compounds were manufactured and commercialized, dominated by the coenzyme nicotinamide adenine dinucleotide (NAD). In many enzymatic tests (also called optical tests), NAD was an obligatory ingredient, because the increase of NADH generated from NAD by an appropriate enzymatic reaction (or coupled reaction) could be used to follow the timecourse of that reaction by spectrophotometry. This was, for the time being, also a helpful technique to determine enzyme levels or metabolites in the clinical laboratory. The methodology had been collected by Hans Ulrich Bergmeyer, a representative of Boehringer Company, who edited a famous compendium (16 volumes) of *Methods in Enzymatic Analysis* (Wiley & Sons).

1.2 Yeast as a Eukaryotic Model System

The unique properties of the yeast, *S. cerevisiae*, among some 1500 yeast species (a subgroup from 700 000 different fungi, which still may expand to over 3000 different yeast species) and its enormous "hidden potential" that has been exploited for many thousands of years made it a suitable organism for research. In fact, yeast was introduced as an experimental organism in the mid-1930s by Hershel Roman (Roman, 1981) and has since received increasing attention. Many researchers realized that yeast is an ideal system in which cell architecture and fundamental cellular mechanisms can be successfully investigated.

Among all eukaryotic model organisms, *S. cerevisiae* combines several advantages. It is a unicellular organism that,

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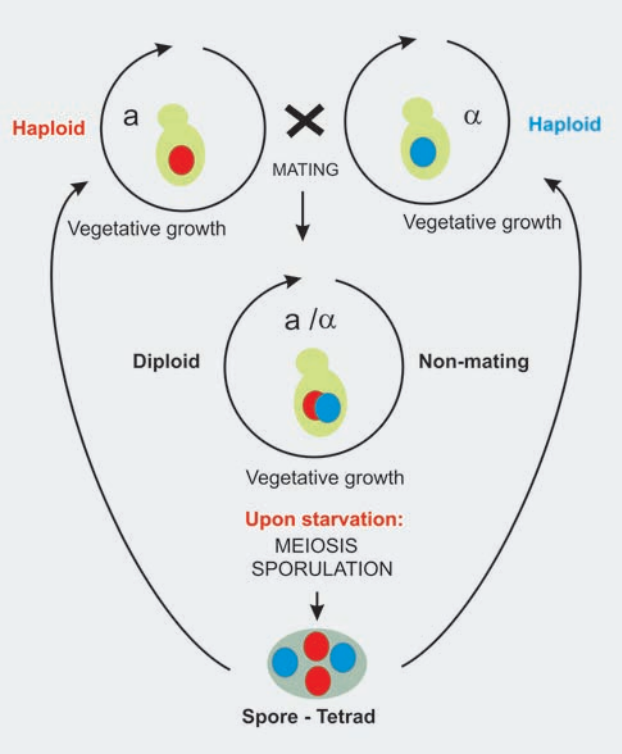

Fig. 1.1 Life cycle of *S. cerevisiae*. Vegetative growth is indicated by the circles.

unlike more complex eukaryotes, can be grown on defined media, giving the investigator complete control over environmental parameters. Yeast is tractable to classical genetic techniques. Both meiotic and mitotic approaches have been developed to map yeast genes (e.g., Mortimer and Schild, 1991). The first genetic map of *S. cerevisiae* was published by Lindegren in 1949 (Lindegren, 1949).

The life cycle of *S. cerevisiae* (Figure 1.1) normally alternates between diplophase and haplophase. Both ploidies can exist as stable cultures. In heterothallic strains, haploid cells are of two mating-types, **a** and α. Mating of **a** and α cells results iin **a**/α diploids that are unable to mate, but can undergo meiosis. The four haploid products derived from meiosis of a diploid cell are contained within the wall of the mother cell (the ascus). Digestion of the ascus and separation of the spores by micromanipulation yields the four haploid meiotic products. Analysis of the segregation patterns of different heterozygous markers among the four spores constitutes the "tetrad analysis" and reveals the linkage between two genes (or between a gene and its centromere). It was mainly Mortimer and his colleagues who undertook the considerable task of collecting and editing all of the genetic data accumulating in diverse laboratories (Mortimer and Hawthorne, 1966), up to the point when genetic maps could be replaced by physical maps. Prior to the start of the Yeast Genome Sequencing Project in 1989 (*cf.* Chapter 12), some 1200 genes had been mapped to the 16 yeast chromosomes, most of them attributable to particular gene functions and others to particular phenotypes only.

During molecular biology's infancy, around the late 1950s, yeast became a convenient organism to be used for the mass preparation of biological material in sufficient quantity or the mass production of other biological compounds. Yeast has a generation time of around 80 min and mass production of cells is easy. Simple procedures for the isolation of high-molecular-weight DNA, ribosomal DNA, mRNA, and tRNA were at hand. It was possible to isolate intact nuclei or cell organelles such as intact mitochondria (maintaining respiratory competence). Eventually, yeast also gained a leading position in basic molecular research. The possibility to apply genetics and molecular methods to an organism at the same time made yeast such a successful a model system. It was the technical breakthrough of yeast transformation (Beggs, 1978; Hinnen, Hicks, and Fink, 1978) that could be used in reverse genetics and for the characterization of many yeast genes that essentially fostered the enormous growth of yeast molecular biology.

The elegance of yeast genetics and the ease of manipulation of yeast substantially contributed to the fact that functions in yeast were studied in great detail using biochemical approaches. A large variety of protocols for genetic manipulation in yeast became available (e.g., Campbell and Duffus, 1988; Guthrie and Fink, 1991; Johnston, 1994). High-efficiency transformation of yeast cells was achieved, for example, by the lithium acetate procedure (Ito *et al.*, 1983) or by electroporation. A large variety of vectors have been designed to introduce and to maintain or express recombinant DNA in yeast cells (e.g., Guthrie and Fink, 1991; Johnston, 1994). The ease of gene disruptions and single-step gene replacements is unique in *S. cerevisiae*, and offered an outstanding advantage for experimentation. Further, a large number of yeast strains carrying auxotrophic markers, drug resistance markers, or defined mutations became available. Culture collections are maintained, for example, at the Yeast Genetic Stock Center (YGSC) and the American Type Culture Collection (ATCC).

The wealth of information on metabolic pathways and the characterization of the enzymes involved in biochemical processes, such as carbon, nitrogen, or fatty acid metabolism, as well as the underlying regulatory circuits and signal transduction mechanisms (e.g., roles of cAMP, inositol phosphates, and protein kinases), has been gathered by numerous yeast researchers. For cytology, studies on yeast contributed to the knowledge of mechanisms in mitosis and meiosis, biogenesis of organelles (such as endosomes, Golgi apparatus, vacuoles, mitochondria, peroxisomes, or nuclear structures), as well as cytoskeletal structure and function. Major contributions came from investigations into nucleic acid and genome structure, protein traffic and secretory pathways, mating-type switching phenomena, mechanisms of recombination, control of the cell cycle, control of gene expression and the involvement of chromatin structure, functions of oncogenes, or stress phenomena. There is too little space here to describe all the achievements made through "classical" approaches and the reader is referred to

detailed collections of articles in standard books (Strathern, Hicks, and Herskowitz, 1981; Broach, Pringle, and Jones, 1991; Guthrie and Fink, 1991).

The success of yeast as a model organism is also due to the fact, which was not fully anticipated earlier than some 20 years ago (Figure 1.2), that many basic biological structures and processes have been conserved from yeast to mammals and that corresponding genes can often complement each other. In fact, a large variety of examples provide evidence that substantial cellular functions are also highly conserved from yeast to mammals.

It is not surprising, therefore, that in those years yeast had again reached the forefront in experimental molecular biology. When the sequence of the entire yeast genome became amenable to thorough analysis, the wealth of information obtained in this project (Goffeau *et al.*, 1996; Goffeau *et al.*, 1997) turned out to be useful as a reference against which sequences of human, animal, or plant genes and those of a multitude of unicellular organisms under study could be compared. Moreover, the ease of genetic manipulation in yeast still opens the possibility to functionally dissect gene products from other eukaryotes in this system.

As it is extremely difficult to follow the contributions of yeast to molecular biology in a strictly chronological sequence *in toto*, I prefer to select particular fields of interest in which the yeast system has served to arrive at fundamental observations valid for molecular and cell biology in general.

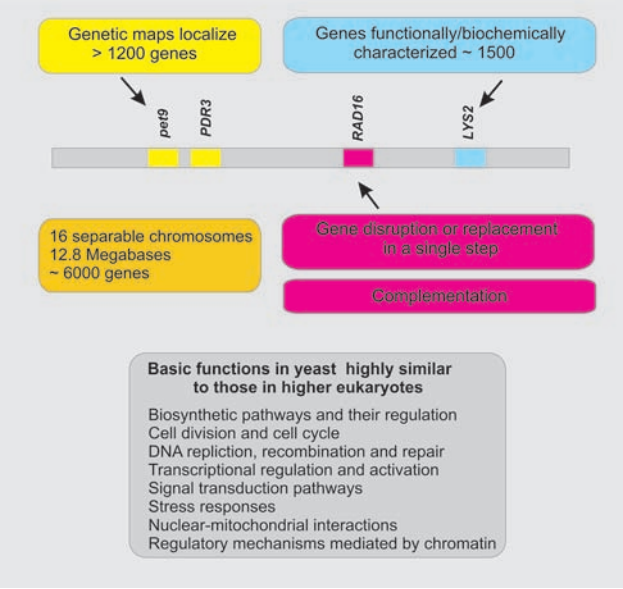

Fig. 1.2 Yeast around the start of the Yeast Genome Sequencing Project.

Summary

- There is no doubt that yeast, *S. cerevisiae*, is one of the oldest domesticated organisms. It has served mankind for thousands of years for baking bread, and making beer and wine. We owe a first glimpse of its nature to van Leeuwenhoek's microscopic description at the end of the seventeenth century. Still, the capability of yeast of fermenting sugar remained a mystery until the middle of the nineteenth century when fermentation could be correlated with yeast metabolism. Indeed, the expression "enzymes" describing the cellular compounds involved in this process is derived from this organism (*en zymi* = in yeast).

- Around 1930, it was recognized that yeast represents an ideal system to investigate cell architecture and fundamental cellular mechanisms, successfully competing with other model organisms such as *Drosophila* or *Neurospora*. Yeast combines several advantages: it has a propagation time comparable to bacterial cells and can be used for mass production of material, it is a unicellular eukaryote that can be grown on defined media, and it is easily tractable to classical genetic analysis including mutational analysis, thus allowing genetic mapping. No wonder then that yeast qualified as a model organism to study metabolic pathways by biochemical and genetic approaches at the same time. Another benefit offered by the yeast system was the possibility to isolate its subcellular components in sufficient quantity and to dissect their functional significance.

- As soon as molecular approaches became available in the mid-1950s, they were successfully applied to yeast. Finally, with the deciphering of its complete genome sequence in 1996, yeast became the first eukaryotic organism that could serve as a model for systematic functional analysis, and as a suitable reference for human, animal, or plant genes and those of a multitude of unicellular organisms. In fact, these comparisons provided evidence that substantial cellular functions are highly conserved from yeast to mammals.

Further Reading

Goffeau, A., Barrell, B.G., Bussey, H. *et al.* (1996) Life with 6000 genes. *Science*, **274**, 546, 563–567 (review).

Hartwell, L.H. (2002) Yeast and cancer. *Nobel Lecture Bioscience Reports*, **22**, 373–394. http://nobelprize.org/nobel_prizes/medicine/laureates/2001/hartwell-lecture.html.

2 Yeast Cell Architecture and Functions

2.1 General Morphology

Cell structure and appearance. Yeast cells exhibit great diversity with respect to cell size, shape, and color. Even individual cells from a pure strain of a single species can display morphological heterogeneity. Additionally, profound alterations in individual **cell morphology** will be induced by changing the physical or chemical conditions at growth. Yeast cell size varies widely – some yeasts may be only 2–3 μm in length, while other species may reach lengths of 20–50 μm. Cell width is less variable at about 1–10 μm. Under a microscope, *Saccharomyces cerevisiae* cells appear as ovoid or ellipsoidal structures, surrounded by a rather thick cell wall (Figure 2.1). Mean values for the large diameter range between 5 and 10 μm, and for the small diameter between 1 and 7 μm. Cell size in brewing strains is usually bigger than that in laboratory strains. Mean cell size of *S. cerevisiae* also increases with age.

With regard to cell shape, many yeast species are ellipsoidal or ovoid. Some, like the *Schizosaccharomyces*, are cylindrical with hemispherical ends. *Candida albicans* and *Yarrowia lipolytica*, for example, are mostly filamentous (with pseudohyphae and septate hyphae). There are also spherical yeasts (like *Debaryomyces* species) or elongated forms (with many yeasts depending on growth conditions).

In principle, the status of *S. cerevisiae* as a eukaryotic cell is reflected by the fact that similar macromolecular constituents are assembled into the structural components of the cell (Table 2.1). There are, however, some compounds that do not occur in mammalian cells or in cells of other higher eukaryotes, such as those building the rigid cell wall or storage compounds in yeast.

For a better understanding of what I will discuss in the following sections, Figure 2.2 presents a micrograph of a dividing yeast cell, indicating some of its major components and organelles. We will deal with the yeast envelope, the cytoplasm, and the cell skeleton, and briefly touch upon the nucleus. The major genetic material distributed throughout the 16 chromosomes residing within the nucleus and other genetic elements, such as the nucleic acids, the retrotransposons, and some extrachromosomal elements, are considered later in Chapter 5. Section 2.5 presents an overview of other yeast cellular structures.

Preparations to view cells. Unstained yeast cells can only be visualized poorly by **light microscopy**. At 1000-fold magnification, it may be possible to see the yeast vacuole and cytosolic inclusion bodies. By using phase-contrast microscopy, together with appropriate staining techniques, several cellular structures become distinguishable. Fluorochromic dyes (*cf.* Table 2.2) can be used with fluorescence microscopy to highlight features within the cells as well as on the cell surface (Pringle *et al.*, 1991).

The range of cellular features visualized is greatly increased, when monospecific antibodies raised against structural proteins are coupled to fluorescent dyes, such as fluorescein isothiocyanate (FITC) or Rhodamine B.

Flow cytometry has several applications in yeast studies (Davey and Kell, 1996). For example, fluorescence-activated cell sorting (FACS) can monitor yeast cell cycle progression, when cell walls are labeled with concanavalin A conjugated to FITC and cell protein with tetramethylrhodamine isothiocyanate (TRITC). These tags enable us to collect quantitative information on the growth properties of individual yeast cells as they progress through their cell cycle.

A very convenient tool to localize and even to follow the movement of particular proteins within yeast cells is the use of the **Green Fluorescent Protein (GFP)** from the jellyfish (*Aequorea victoria*) as a reporter molecule (Prasher *et al.*, 1992), as well as several derivatives of GFP with fluorescence spectra shifted to other wavelengths (Heim *et al.*, 1994; Heim, Cubitt, and Tsien, 1995). Fusions of genes of interest with the fluorescent protein gene (N- or C-terminal) also allow us to follow the expression and destiny of the fusion proteins followed by fluorescence microscopy (Niedenthal *et al.*, 1996; Wach *et al.*, 1997; Hoepfner *et al.*, 2000; see also Chapter 4).

Organelle ultrastructure and macromolecular architecture can only be obtained with the aid of electron microscopy, which in scanning procedures is useful for studying cell topology, while ultrathin sections are essential in transmission electron microscopy to visualize intracellular fine structure (Streiblova, 1988). Atomic force microscopy can be applied to uncoated, unfixed cells for imaging the cell

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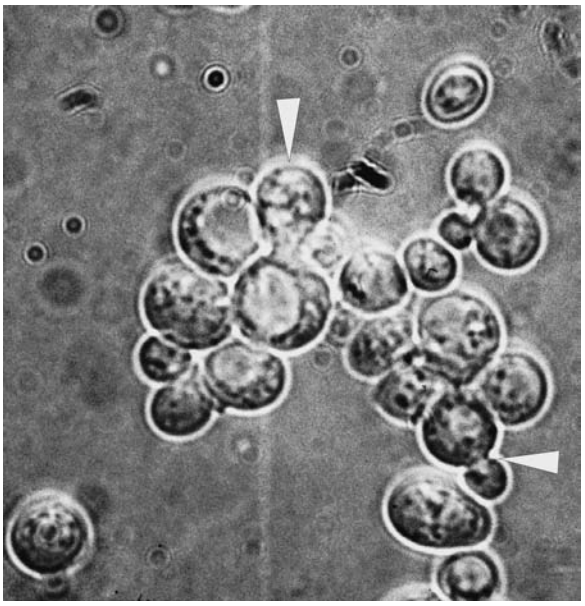

Fig. 2.1 Cells of *S. cerevisiae* under the microscope. The white arrows point to dividing cells.

Table 2.1 Classes of macromolecules in *S. cerevisiae*.

Class	Category	Major compounds
Proteins	structural	actin, tubulin (cytoskeleton)
		histones (H2A, H2B, H3, H4, H1)
		ribosomal proteins
	hormones	pheromones α and **a**
	functional	enzymes and factors
		transporters
		signaling receptors
		motor proteins (myosins, kinesins, dynein)
Glycoproteins	cell wall components	mannoproteins
	enzymes	many functional enzymes (e.g., invertase)
Polysaccharides	cell wall components	glucan, mannan, chitin
	capsular components	glucan, mannan, chitin
	storage	glycogen, trehalose
Polyphosphates	storage	polyphosphate in vacuole
Lipids	structural	free sterols in membranes
	storage	lipid particles (sterol esters and triglycerides)
	functional	phosphoglyceride derivatives, free fatty acids
Nucleic acids	DNA	genomic DNA (80%), mitochondrial DNA (10–20%)
	RNA	rRNA (80%), mRNA (5% cytosolic, ER, mitochondria), tRNAs, snRNAs, snoRNAs

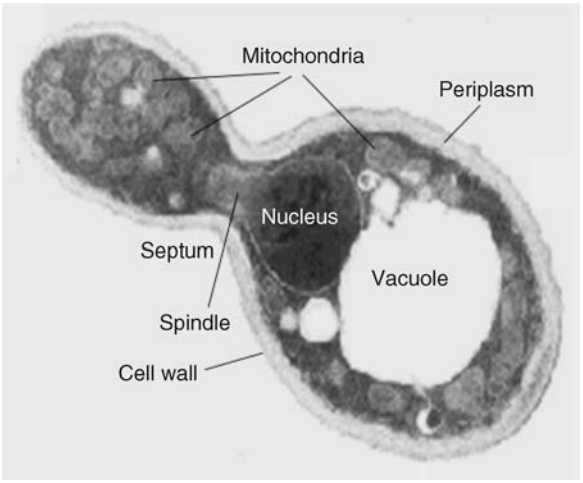

Fig. 2.2 Micrograph of a dividing yeast cell.

Table 2.2 Some structure-specific dyes for yeast cells.

Dye	Structures visualized	Comments
Methylene blue	whole cells	nonviable cells stain blue
Aminoacridine	cell walls	indicator of surface potential
F-C ConA	cell walls	binds specifically to mannan
Calcofluor white	bud scars	chitin in scar fluoresces
DAPI	nuclei	DNA fluoresces
DAPI	mitochondria	mitochondria fluoresce pink-white
Neutral red	vacuoles	vacuoles stain red-purple
Iodine	glycogen deposits	glycogen stained red-brown
Rhodamine	mitochondria	

DAPI, 4,6-diamidino-2-phenylindole.

surfaces of different yeast strains or of cells under different growth conditions (De Souza Pereira *et al.*, 1996).

A most convenient method to mark specific cellular structures or compartments is to check for particular marker enzymes that occur in those structures (Table 2.3).

2.2 Cell Envelope

In *S. cerevisiae*, the **cell envelope** occupies about 15% of the total cell volume and plays a major role in controlling the osmotic and permeability properties of the cell. Looking from the inside out, the yeast cytosol is surrounded by the plasma membrane, the periplasmic space, and the cell wall. Structural and functional aspects of the yeast cell envelope have attracted early interest (Phaff, 1963) because – like the cell envelope of fungi in general – it differs from bacterial envelopes and from those of mammalian cells. A peculiarity of yeast is that once the cell has been depleted of its cell wall,

Table 2.3 Marker enzymes for isolated yeast organelles.

Organelle	Compartments	Marker enzyme
Cell wall	periplasm	invertase
	secretory pathway	acid phosphatase
Plasma membrane		vanadate-sensitive ATPase
Cytosol		glucose-6-phosphate dehydrogenase
Nucleus	nucleoplasm	RNA polymerase
	nuclear envelope	transmission electron microscopy
ER	light microsomal fraction	NADPH: cytochrome *c* oxidoreductase
Vacuole	membrane	α-mannosidase
	sap	protease A and B
Golgi apparatus		β-glucan synthase, mannosyltransferase
Mitochondrion	matrix	aconitase, fumarase
	intermembrane space	cytochrome *c* peroxidase
	inner membrane	cytochrome *c* oxidase
	outer membrane	kynurenine hydroxylase
Peroxisome		catalase, isocitrate lyase, flavin oxidase

protoplasts are generated that are able to completely regenerate the wall (Necas, 1971).

2.2.1 Cell Wall

Yeast cell wall. The outer shell is a rigid structure about 100–200 nm thick and constituting about 25% of the total dry mass of the cell (Figure 2.3). The cell wall is composed of only four classes of macromolecules: highly glycosylated glycoproteins ("mannoproteins"), two types of β-glucans, and chitin. The composition of the cell wall is subject to considerable variation according to growth conditions, and the biosynthesis of the single compounds is highly controlled both in space and in time. The literature that has accumulated on these issues has grown so voluminous that reference is given here to only a few review articles (Klis, 1994; Lipke and Ovalle, 1998; Cabib *et al.*, 2001). Details of cell wall synthesis during yeast growth and budding, as well as septum formation (Cid *et al.*, 1995; Cabib *et al.*, 1997; Cabib *et al.*, 2001; Smits, van denEnde, and Klis, 2001), are considered below.

By treatment with lytic enzymes in the presence of osmotic stabilizers, the yeast cell wall can be removed without harming viability or other cellular functions. These "naked" cells are called **spheroplasts**. The cell wall will regenerate and this process has been used to study aspects of cell wall biosynthesis. Spheroplasts are amenable to intergeneric and intrageneric cell fusions; such hybrids are valuable instruments in genetic studies and possess a valuable biotechnological potential. A cell wall protein that contains a putative glycosylphosphatidylinositol (GPI)-attachment site, Pst1p, is secreted by regenerating protoplasts. It is upregulated by activation of the cell integrity pathway, as mediated by Rlm1p, as well as upregulated by cell wall damage via disruption of the *FKS1* gene, representing the catalytic subunit of glucan synthase (*cf.* Chapter 3).

Yeast cell aggregation. A phenomenon of particular importance in brewing is flocculation. It is based on asexual cellular aggregation when cells adhere, reversibly, to one another, which leads to the formation of macroscopic flocs sedimenting out of suspension. Traditionally, brewing yeast strains are distinguished as highly flocculent bottom yeasts (used for lager or Pilsner fermentations) or weakly flocculent top yeasts (used for ale fermentations or, in Germany, to prepare "top-fermented" beers). Although flocculation is far from being completely understood, it appears that the phenomenon is due to specific cell wall lectins in yeast (so-called flocculins) – surface glycoproteins capable of directly binding mannoproteins of adjacent cells. Yeast flocculation is genetically determined by the presence of different *FLO* genes. One such protein is Flo1p, a lectin-like cell-surface protein that aggregates cells into "flocs" by binding to mannose sugar chains on the surfaces of other cells. Both the

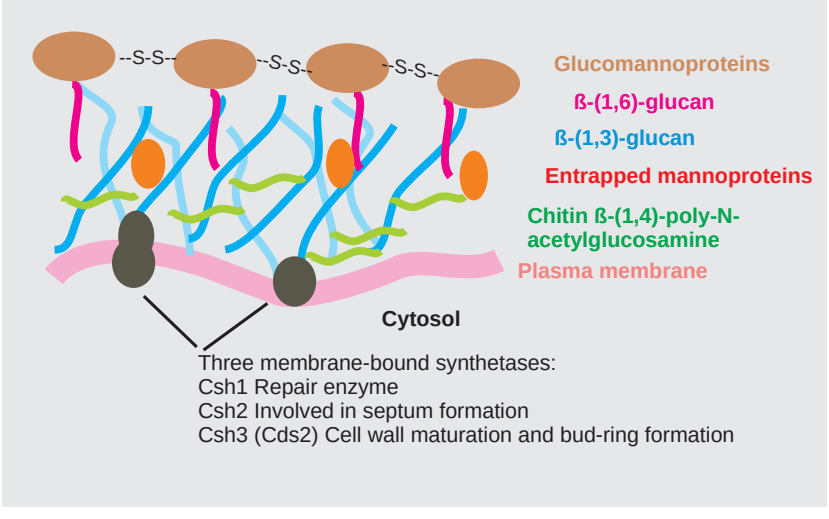

Fig. 2.3 Schematic representation of the yeast cell wall.

phenotypic characterization of *FLO5* strains and the sequence similarity between Flo1p and Flo5p suggest that Flo5p is also a mannose-binding lectin-like cell surface protein.

As the yeast cell wall is involved in sexual **agglutination**, some attention has been given to this particular aspect (Lipke and Kurjan, 1992). **a-** and α-cells can be distinguished by their agglutinin proteins. The anchorage subunit of **a**-agglutinin, Aga1p, is a highly *O*-glycosylated protein with an N-terminal secretion signal and a C-terminal signal for the addition of a GPI anchor (*cf.* Section 3.4.3.2). Linked to the anchoring subunit by two disulfide bonds is the adhesion subunit Aga2p. The α-agglutinin of α-cells is Sag1p. It binds to Aga1p during agglutination; its N-terminus is homologous to members of the immunoglobulin superfamily, containing binding sites for **a**-agglutinin, while the C-terminus is highly glycosylated and harbors GPI anchor sites.

The cell wall as a target for the defeat of mycoses. Similarly, several peculiarities of fungal cell wall synthesis such as the occurrence of ergosterol have led to the development of strategies for their inhibition as a means to defeat severe **mycoses** (Gozalbo *et al.*, 1993). A more recent brief account is given in an article by Levin (2005) describing cell wall integrity regulation in *S. cerevisiae*, which is considered a good model for the development of safe and effective antifungal agents. At present, effective antifungal therapy is very limited and dominated by the azole class of ergosterol biosynthesis inhibitors. Members of this class of antifungals are cytostatic rather than cytotoxic and therefore require long therapeutic regimens. The antifungal drugs can be applied to the major opportunistic human pathogens (*Candida* species, *Aspergillus fumigatus*, and *Cryptococcus neoformans*) causing systemic infections among immunocompromised patients. As this population has grown over the past three decades due to HIV infection, cancer chemotherapy, and organ transplants, and the number of life-threatening systemic fungal infections has increased accordingly, there is a need to develop safe, cytotoxic antifungal drugs (*cf.* Chapter 14).

2.2.2 Plasma Membrane

Like other biological membranes, the surface **plasma membrane** of yeast can be described as a lipid bilayer, which harbors proteins serving as cytoskeletal anchors, and enzymes for cell wall synthesis, signal transduction, and transport. The *S. cerevisiae* plasma membrane is about 7.5 nm thick, with occasional invaginations protruding into the cytoplasm. The lipid components comprise mainly phospholipids (phosphatidylcholine, phosphatidylethanolamine, and minor proportions of phosphatidylinositol, phosphatidylserine, and phosphatidylglycerol) as well as sterols (principally ergosterol and zymosterol). Like the cell wall, the plasma membrane changes both structurally and functionally depending on the conditions of growth.

The primary functions of the yeast plasma membrane are:

i) Physical protection of the cell.
ii) Control of osmotic stability.
iii) Control of cell wall biosynthesis.
iv) Anchor for cytoskeletal compounds.
v) Selective permeability barrier controlling compounds that enter or that leave the cell. Of prime importance in active transport of solutes is the activity of the plasma membrane proton-pumping ATPase (see Section 5.6.1).
vi) Transport-related functions in endocytosis and exocytosis.
vii) Location of the components of signal transduction pathways.
viii) Sites of cell–cell recognition and cell–cell adhesion (Van der Rest *et al.*, 1995).

A comprehensive coverage of the lipids and the yeast plasma membrane, as well as on the biogenesis of the cell wall, can be found in a book by Dickinson and Schweitzer (2004).

The **periplasmic space** (Arnold, 1991) is a thin (35–45 Å), cell wall-associated region external to the plasma membrane. It comprises mainly secreted proteins that are unable to permeate the cell wall, such as invertase and phosphatase, which catabolize substrates that do not cross the plasma membrane. The unique properties of invertase have inspired its commercial preparation for the confectionary industry. The signal sequences of invertase (*SUC2*) and phosphatase (*PHO5*) have been used in recombinant DNA technology to generate heterologous proteins that can be secreted (Hadfield *et al.*, 1993). Most frequently used for secretion of heterologous proteins is the prepro-α-factor (*MFα1*) (Brake, 1989) (*cf.* Section 4.2.2.3).

2.3 Cytoplasm and Cytoskeleton

2.3.1 Yeast Cytoplasm

Like in all other cellular organisms, the **yeast cytoplasm** is the site for many cellular activities and the space for intracellular traffic. In yeast, it is an aqueous, slightly acidic (pH 5.2) colloidal fluid that contains low- and intermediate-molecular-weight weight compounds, such as proteins, glycogen, and other soluble macromolecules. Larger macromolecular entities like ribosomes, proteasomes, or lipid particles are suspended in the cytoplasm. The cytosolic (non-organellar) enzymes include the glycolytic enzymes, the fatty acid synthase complex, and the components and enzymes for protein biosynthesis. Many functions essential for cellular integrity are localized to the cytoplasm (e.g., the components that form and control the cytoskeletal scaffold).