

2 Yeast Cell Architecture and Function

2.1 General Cellular Characteristics of Yeast

Yeast cells exhibit great diversity with respect to **cell size**, shape and colour. Even individual cells from a particular yeast strain of a single species can display morphological and colour heterogeneity. This is mainly due to alterations of physical and chemical conditions in the environment. Among different yeast species, cell size may vary widely. In the following we will concentrate on *S. cerevisiae*. More detailed information and references on yeast cytology can be found in [Walker, 1998].

S. cerevisiae cells are generally ellipsoidal in shape ranging from 5 to 10 μm at the large diameter and 1 to 7 μm at the small diameter. Mean cell volumes are 29 or 55 μm^3 for a haploid or a diploid cell, respectively; cell size increases with age.

Macromolecular constituents of yeast comprise proteins, glycoproteins, polysaccharides, polyphosphates, lipids, and nucleic acids (Table 2-1).

Table 2-1: Classes of molecules encountered in yeast.

| Class of Macromolecule | Categories | Major Components |
|------------------------|----------------------|---|
| Proteins | Structural | actin, tubulin (cytoskeleton) histones (H2A, H2B, H3, H4, no H1) ribosomal proteins |
| | Hormones | α and a pheromones |
| | Enzymes | |
| Glycoproteins | Cell wall components | mannoproteins |
| | Enzymes | functional enzymes (invertase) |
| Polysaccharides | Cell wall components | glucan, mannan, chitin |
| | Capsular components | |
| | Storage | glycogen, trehalose |
| Polyphosphates | Storage | polyphosphate in vacuole |
| Lipids | Structural | free sterols in membranes |
| | Storage | lipid particles (sterol esters and triglycerides) |
| Nucleic acids | Functional | phosphoglyceride derivatives, free fatty acids |
| | DNA | genomic DNA (80%); mitochondrial (10-20%) |
| | RNA | rRNA (80%); mRNA (5% cytosol, ER, mitochondria), tRNAs, snRNAs |

2.2 Cytological Methods

Unstained yeast cells can hardly be visualized by light microscopy. At 1000-fold magnification, it may be possible to see the yeast vacuole and cytosolic inclusion bodies. By phase-contrast microscopy, together with appropriate staining techniques, several cellular structures can be distinguished. Fluorochromic dyes can be used with fluorescence microscopy to highlight features within the cells as well as on the cell surface [Pringle et al., 1991].

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, and several derivatives of GFP with fluorescence spectra shifted to other wavelengths. Fusions of genes of interest with the GFP gene (N- or C-terminal) also allow to follow the expression and destiny of the fusion proteins followed by fluorescence microscopy [Niedenthal et al., 1996; see also chapter 4].

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.

Table 2-2: Structure-specific dyes for yeast cells.

| Dye | Structures visualized | Comments |
|-------------------------------------|-----------------------|-----------------------------------|
| Methylene Blue | Whole cells | Non-viable 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 (4,6-diamidino-2-phenylindole) | Nuclei | DNA fluoresces (Figure 2-1) |
| Neutral red | Vacuoles | Vacuoles stain red-purple |
| Iodine | Glycogen deposits | Glycogen stained red-brown |
| DAPI | Mitochondria | Mitochondria fluoresce pink-white |
| Rhodamine | Mitochondria | |

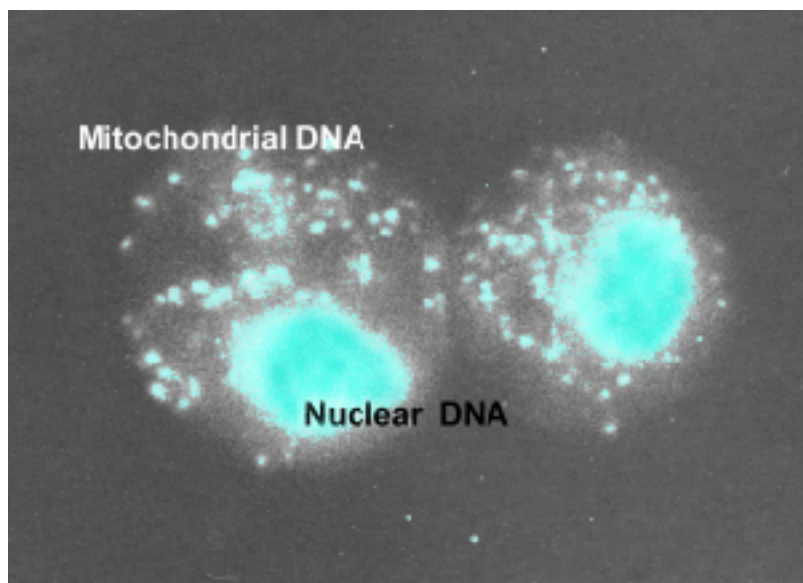


Figure 2-1: DAPI-staining of yeast cells.

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

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. Atomic force microscopy can be applied to uncoated, unfixed cells of imaging the cell surfaces of different yeast strains or of cells under different growth conditions.

2.3 Yeast Cell Organelles and Compartments

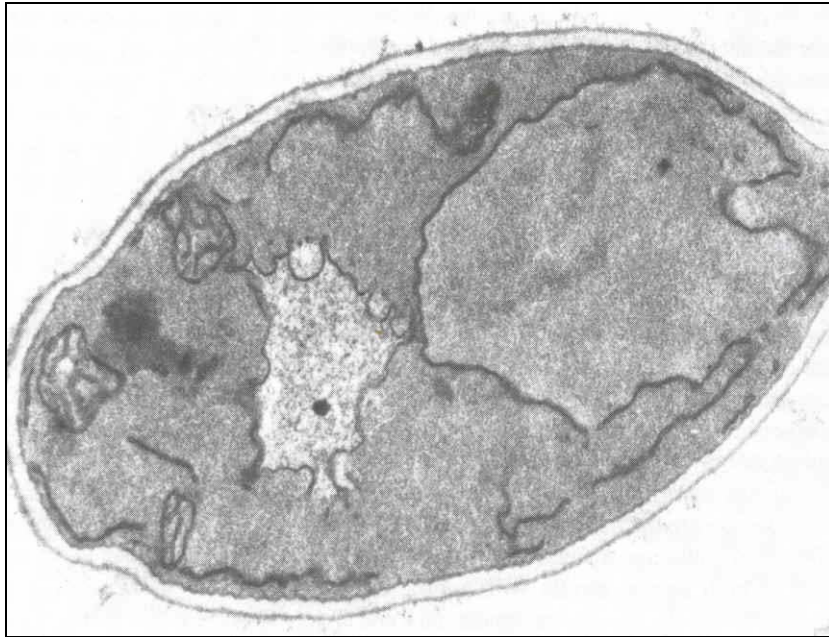


Figure 2-2: Thin section of a yeast cell.

In an idealized **yeast cell** (Figure 2-2), the following ultrastructural features can be observed (Figure 2-3): cell wall; periplasm; plasma membrane; invagination; bud scar; cytosole; nucleus; mitochondrion; ER, endoplasmic reticulum; Golgi apparatus; secretory vesicles; vacuole; peroxisome. Obviously, yeast cells share most of the structural and functional features of higher eukaryotes, which has rendered yeast an ideal model for eukaryotic cell biology. In contrast to mammalian cells, peculiarities of yeast cells are that they are surrounded by a rigid cell wall and develop birth scars during cell division; the vacuole corresponds to lysosomes in higher cells. Table 2-3 offers a list of marker enzymes that can be used to specifically identify these structures.

Table 2-3: Organelles and compartments in a yeast cell.

| Organelle/Compartment | | Marker enzyme |
|-----------------------|---------------------------------|---|
| Cell wall | periplasm secretory | Invertase Acid phosphatase |
| Plasma membrane | | Vanadate-sensitive ATPase |
| Cytosole | | G-6-PDH |
| Nucleus | nucleoplasm nuclear envelope | RNA polymerase transmission EM |
| Endoplasmic reticulum | light microsomal fraction | NADPH:cytochrome c oxidoreductase |
| Vacuole | membrane sap | α -Mannosidase Protease A and B |
| Golgi apparatus | | β -Glucan synthase; |

| | | |
|---------------|---|---|
| Mitochondrion | matrix intermembrane space inner membrane outer membrane | mannosyltransferase Aconitase; fumarase Cytochrome c peroxidase Cytochrome c oxidase Kynurenine hydroxylase |
| Peroxisome | | Catalase; isocitrate lyase; flavin oxidase |

Subcellular structures from yeast cells can be isolated from protoplasts or from intact cells by breaking the cell wall prior to differential centrifugation (see chapter 4: Techniques).

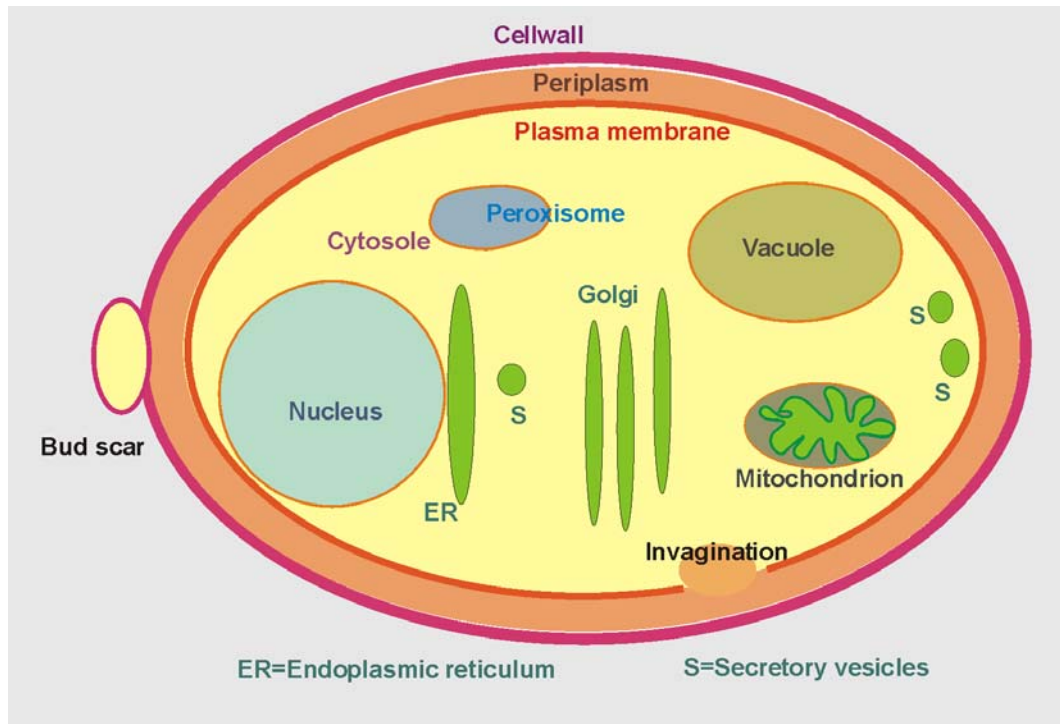


Figure 2-3: Scheme of organelles and compartments in a yeast cell.

2.3.1 Cell Envelope

The yeast **cell envelope** is a protecting capsule, consisting of three major constituents (inside out): the plasma membrane, the periplasmic space, and the cell wall. In *S. cerevisiae*, the cell envelope takes ca. 15% of the total cell volume and has a major role in controlling the osmotic and permeability properties of the cell.

The **plasma membrane** is about 7 nm thick, with some invaginations into the cytosole. Like other membranes, it is a lipid bilayer with proteins inserted into this layer or traversing it as transmembrane proteins of various functions. The lipid composition comprises mainly phosphatidylcholine and phosphatidylethanolamine, with minor proportions of phosphatidylinositol, phosphatidylserine or phosphatidyl-glycerole, as well as sterols, mainly ergosterol and zymosterol. Yeast membrane proteins include the following categories: (i) cytoskeleton anchors; (ii) enzymes for cell wall synthesis; (iii) proteins for transmembrane signal transduction; (iv) proteins for solute transport (permeases, channels, ATPases); (v) transport facilitators, such as the ABC (ATP binding cassette) proteins involved in multidrug transport.

Thus the primary functions of the yeast cell membrane is to provide selective permeability, i.e. to control what enters and what leaves the cytosole. Most important is the role of membrane proteins in regulating yeast nutrition, such as uptake of carbohydrates, nitrogenous compounds or ions, and the extrusion of molecules hazardous to the cell. Other important aspects include exo- and endocytosis of cargo molecules, stress responses, and sporulation.

The yeast **periplasm** is a thin (35-45 Å), cell wall associated region external to the plasma membrane and internal to the cell wall. It mainly contains secreted proteins (mannoproteins) that are unable to permeate the cell wall, but fulfill essential functions in hydrolysing substrates that do not cross the plasma membrane: invertase converts sucrose into glucose and fructose; acid phosphatase catalyzes the liberation of free phosphate from organic compounds.

2.3.2 Cell Wall

The **wall of a yeast cell** is a remarkably thick (100 to 200 nm) envelope (Figures 2-4 and 2-5), which contains some 15 to 25% of the dry mass of the cell. Major structural constituents of the cell wall are polysaccharides (80-90%), mainly glucans and mannans, with a minor percentage of chitin. Glucans (both β -2,6 and β -1,3-linked glucans are represented) provide strength to the cell wall, forming a microfibrillar network. Mannans are present as an α -1,6-linked inner core with α -1,2- and α -1,3 side chains. Chitin is a polymer of N-acetylglucosamine representing only 2-4% of the cell wall and mainly located in bud scars. It may be interesting to note that some filamentous growing yeasts, such as *Candida albicans*, have a higher content of chitin, while chitin is absent from many other yeast species. Other components of the cell wall are variable quantities of proteins, lipids, and inorganic phosphate. Preparation of cell walls are described in Zinser and Daum, 1995.

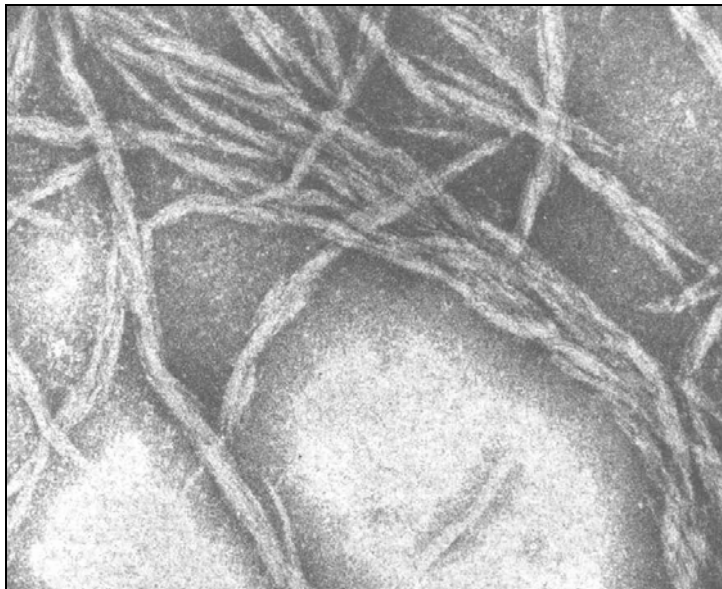


Figure 2-4: Architecture of a yeast cell wall.

Table 2-4: Drugs to treat systemic mycoses.

| Compound | Effects |
|-----------------|--|
| Amphotericin | Complexes with membrane sterols (ergosterols) and leads to cell disruption |
| Fluconazole | Interferes with ergosterol biosynthesis (blocks P450-dependent demethylation step) and leads to accumulation of lanosterol |
| 5-Fluorocytosin | Is deaminated to 5-Fluorouracil Incorporated in RNA, inhibits protein synthesis If converted to 5-Fluorodeoxyuridylate, is incorporated in DNA and inhibits thymidylate synthase |
| Bacilysin | Inhibitor of glucosamin-6-p synthesis from Fructose-6-p and glutamin |

Bud scars are specialized, ring-shaped convex protrusions at the cell surface which remain on the mother cells (of budding yeasts) after cell division and birth of daughter cells (Figure 2-6). The concave indentations remaining on the surface of the daughter cell after budding are called birth scars.

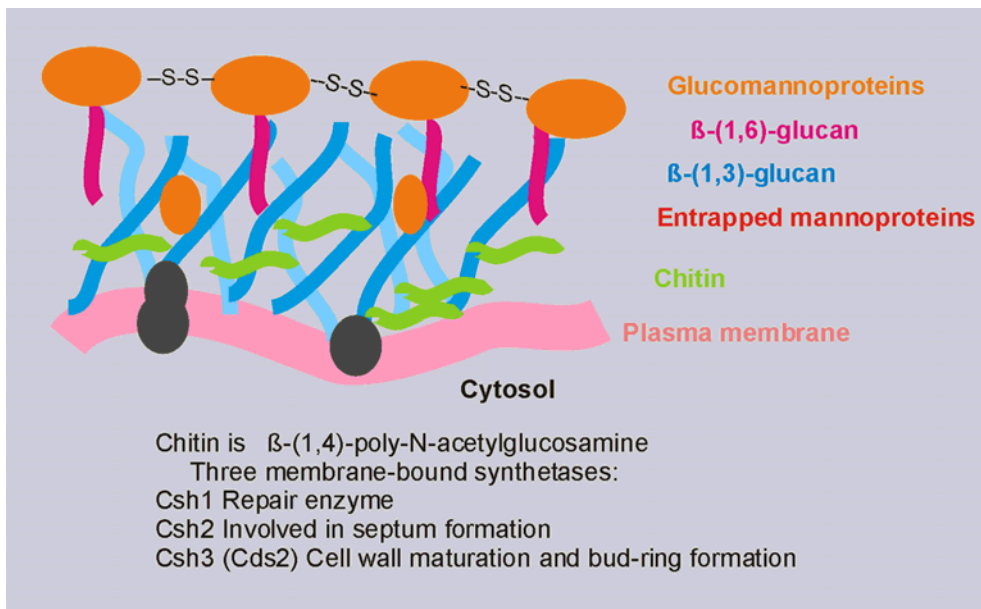


Figure 2-5: Constituents of a yeast cell wall..

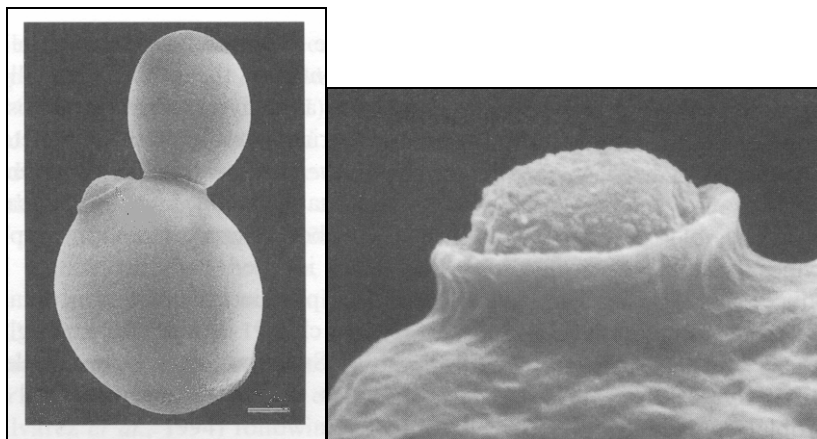


Figure 2-6: The yeast bud.

If yeast cells are treated with lytic enzymes (e.g. *Helicase* from snail digestive juice; *Zymolase* or *Lyticase* from microbial sources) in the presence of osmotic stabilizers, the cell wall is removed giving rise to the formation of **spheroblasts**, which can be visualized as globular structures in the microscope. Spheroblast formation is often used prior to facilitate the isolation of subcellular components. Remarkably, spheroblasts keep the potential of regenerating the cell wall (Figure 2-7).

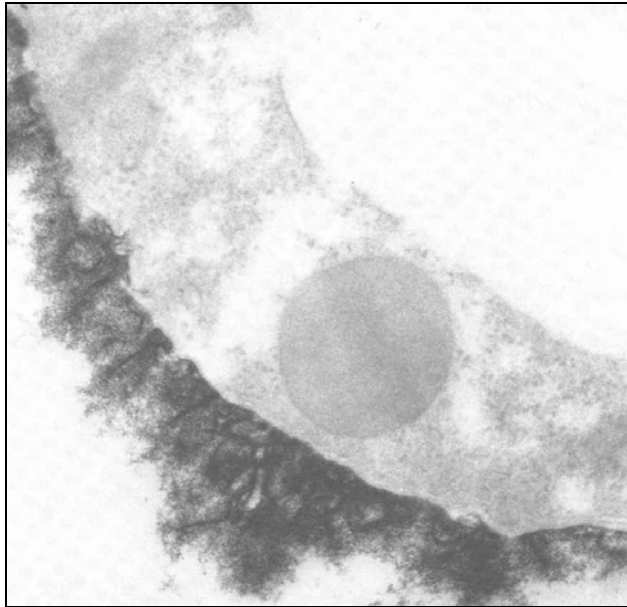


Figure 2-7: Micrograph of a regenerating yeast protoplast.

Flocculation (asexual cell aggregation) is a phenomenon of particular interest in brewing, because cells of brewer's yeast strains tend to aggregate to a different extent. Bottom yeasts, normally used for larger fermentations, show a higher degree of flocculation (i.e. formation of macroscopic flocs by cell adherence) than top yeasts, which are used for making special kinds of beer. Most probably, flocculation is a consequence of the differential expression of flocculins, mannose-specific lectins of yeast cells, which interact with mannose receptors on the cell wall of neighbouring cells.

2.3.3 Cytosol and Cytoskeleton

The yeast **cytoplasm** is an acidic (pH 5.25) colloidal fluid, mainly containing ions and low or intermediate molecular weight organic compounds, and soluble macromolecules (e.g. enzyme proteins, factors, glycogen). The cytosolic enzymes of yeast include those of: (i) the glycolytic pathway, (ii) the fatty acid synthase complex, and (iii) some enzymes for protein biosynthesis. The **cytoskeletal network** guaranteeing internal stability to the cell and providing structural organization comprises the **microtubules** and the **microfilaments**. These are dynamic structures which fulfill their function through regulated assembly and dis-assembly of individual protein subunits. Thus, α and β tubulin monomers polymerize as heterodimers to give microtubules, while globular monomers of G-actin polymerize into double-stranded microfilaments of F-actin. Microtubules and microfilaments are also important in several dynamic processes occurring during mitosis and meiosis, septation, and organelle motility.

The yeast cytoplasm contains several categories of microbodies, which may be distinguished from organellar substructures:

(i) Freely suspended 80S ribosomes (in contrast to ER associated and mitochondrial 60S ribosomes);
(II) Lipid particles, which function as storage particles or may serve in yeast membrane biosynthesis;
(III) Proteasomes, multi-subunit complexes involved in programmed proteolysis of proteins and in other aspects of protein degradation or transport. Because of their importance in cellular metabolism and regulation, the ubiquitin-proteasome pathway will be discussed separately (see chapter 'Specialized Protein Families and Pathways').

2.3.4 Nucleus and Extrachromosomal Elements

The yeast **nucleus** is a round-lobate organelle, some 1.5 μm in diameter. The **nucleoplasm** is separated from the cytosol by a double membrane containing pores between 50 to 100 nm in diameter. At two opposite poles, **spindle pole bodies** (SPBs) are located which are interconnected by continuous intranuclear microtubules and the origins of discontinuous microtubules. On the outer face, the SPBs are connected to cytosolic microtubules. These structural elements play an important role during cell division, cytokinesis and bud formation. In contrast to other eukaryotes, the nuclear membrane in yeast is not dissolved during mitosis.

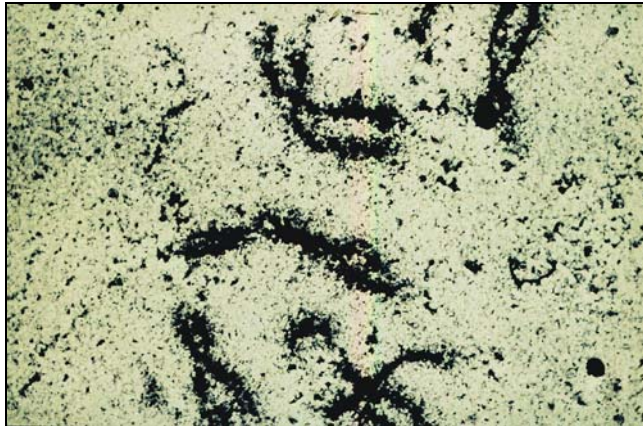


Figure 2-8: EM picture of yeast chromosomes.

Within the nucleus there is a dense region corresponding to the **nucleolus** which disappears during mitosis and reforms during interphase. The major content of the nucleoplasm is represented by the genomic DNA which together with histones and non-histones is organized into chromatin. Yeast **chromosomes** are formed and replicated during mitosis (or meiosis) but behave virtually invisible by microscopic techniques (Figure 2-8). However, pulsed field gel electrophoresis (PFGE) techniques provide convenient tools for chromosome separation and karyotyping (Figure 2-9).

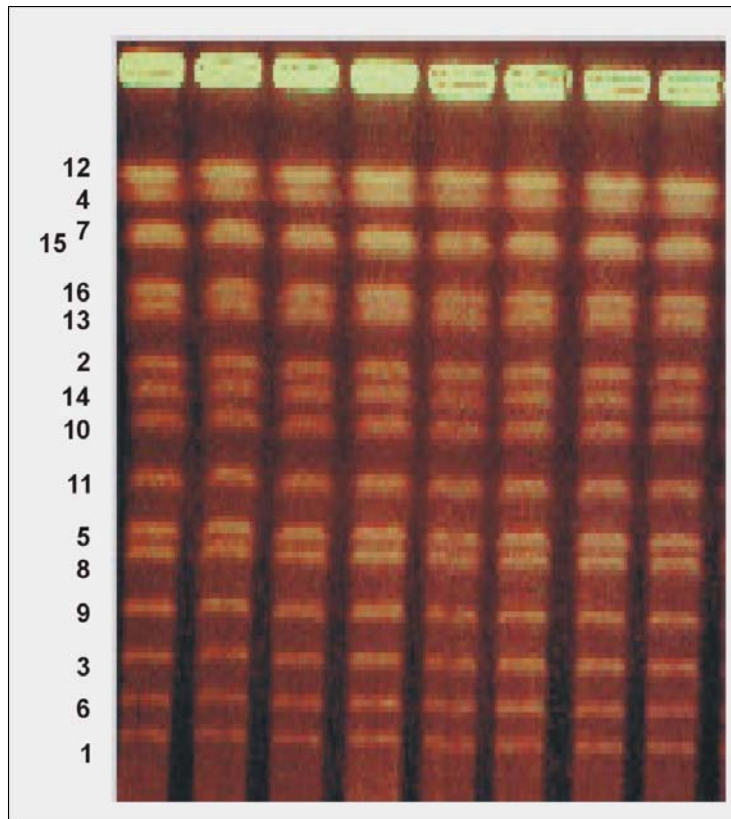


Figure 2-9: Separation of yeast chromosomes by pulsed-field gel electrophoresis.

In addition to the genomic material, yeast nuclei contain the machineries for DNA replication, DNA repair, transcription and RNA processing together with the necessary substrates and regulatory factors, and the resulting (precursor) products, as well as a proportion of the yeast proteasomes.

Furthermore, several non-chromosomal genetic elements may be present in the yeast nucleus [Wickner, 1995].

(i) **2 μ m DNA** is a stably maintained circular DNA plasmid, which replicates exactly once during S phase. These elements can be present in high copy number and have been useful in the construction of cloning vectors in yeast recombinant DNA technology. No functions have yet been attributed to the four genes found in 2 μ m DNA.

(ii) Double-stranded RNA and linear DNA are found in **killer strains** of yeast. They harbour genes for toxins, which will be hazardous to non-killer strains.

(iii) Most interesting extrachromosomal elements are the Ty elements, the only class of retrotransposons found in yeast.

Aspects of most of the afore mentioned subjects (such as mitosis, DNA replication, chromatin and transcription, proteasomes, nuclear transport, and the yeast retroposons) will be detailed in separate chapters.

2.3.5 Secretory System and Vacuoles

As common to all eukaryotes, yeast cells harbor a system of membrane-surrounded **compartments** that are designed for trafficking of proteins within, into and out of the cell. In our fundamental understanding of the underlying processes and their regulation, yeast has contributed as a convenient model system [Pelham et al., 1995].

The **endoplasmic reticulum** (ER) is the site of biosynthesis and modification of proteins that are to be exported. After synthesis on ER-associated polysomes located on the surface of the ER membrane, precursor proteins are translocated into the lumen of the ER, where trimming of the precursors, chaperone-assisted folding and glycosylation of the proteins occur. From the ER, proteins are directed to the **Golgi apparatus** by **vesicles**, which fuse at the cis-side and are exported from the Golgi at the trans-side. In the Golgi further modifications of the proteins by carbohydrate side chains may take place, such as mannosylation. **Retrograde transport** from the Golgi to the ER has been established as a quality control for the exported proteins.

Proteins delivered from the Golgi are directed to different destinations within the cell or to the exterior *via* different secretory vesicles. These destinations include: (i) the vacuole; (ii) the bud region during mitosis targeted by actin-mediated transport; (iii) the plasma membrane; (iv) the periplasm. Naturally, only few proteins are exported to the periplasm and at low abundance. Nevertheless, the signal sequences which are present in these proteins have been fused to recombinant heterologous proteins of therapeutic value, which are then successfully secreted from yeast cells.

The key organelle in yeast involved in intracellular trafficking of proteins is the **vacuole**. It can be viewed as a form of integral component of the intramembranous system. The main role of this lysosome-like compartment is the non-specific proteolytic cleavage of proteins, which involves a variety of intravacuolar lytic enzymes: endopeptidases, aminopeptidases, and carboxypeptidases. Further physiological functions of yeast vacuoles include: storage of basic amino acids, polyphosphates and certain metal ions; homeostasis of cytoplasmic ion concentrations; osmoregulation.

The import of proteins into yeast cells by endocytosis also involves membran-bound vesicles (endocytotic vesicles), which deliver their cargo to the vacuole for proteolytic processing [Riezman, 1993].

2.3.6 Peroxisomes

Peroxisomes perform a variety of metabolic functions in eukaryotic cells. In yeasts, peroxisomes (Figure 2-10) contain several oxidases which serve in oxidative utilization of specific carbon and nitrogen sources. The organelles develop from the small peroxisomes present in glucose-grown cells as a result of rapid synthesis of peroxisomal enzymes, such as catalase and alcohol oxidase. The import of components into peroxisomes will be discussed elsewhere (Chapter: Transport). In *S. cerevisiae*, most of the genes involved in peroxisome biogenesis (PEX genes) have been characterized to date. As yeast mitochondria lack β -oxidation, peroxisomes are the sites of fatty acid degradation (Figure 2-11).

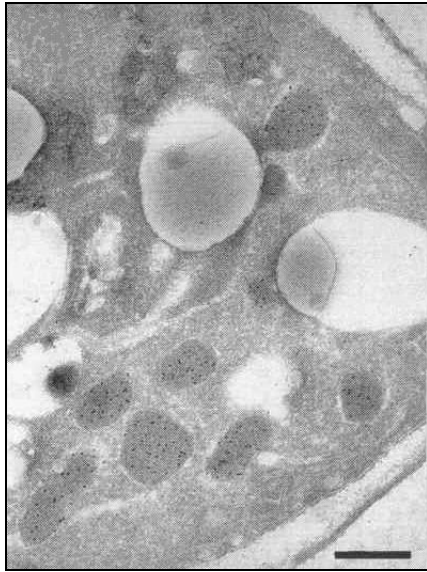


Figure 2-10: Micrograph of yeast peroxisomes.

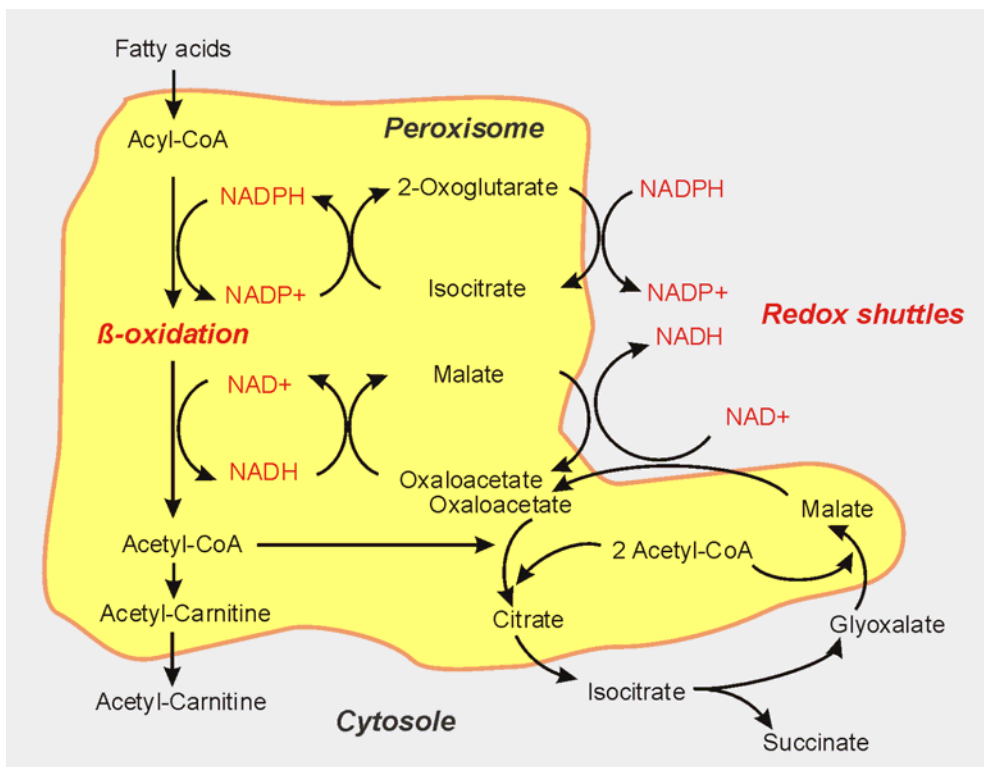


Figure 2-11: Functions of the yeast peroxisome. Yeast lacks the mitochondrial β -oxidation. Acetyl-CoA, NADH and NADPH are produced in peroxisomes.

2.3.7 Mitochondria

Yeast cells contain **mitochondria** which structurally resemble these organelles found in all eukaryotes. Therefore, yeast mitochondria have served as models to intensely study mitochondrial structure, function and biogenesis [Glick and Pon, 1995]. However, yeast mitochondria exhibit a variety of important features which are absent from their counterparts in higher organisms, notably mammalian cells.

General structural characteristics of mitochondria (Figure 2-12) include:

- (i) an outer membrane – containing enzymes involved in lipid metabolism,
- (ii) the intermembrane space
- (iii) an inner membrane – containing NADH and succinate dehydrogenases, the components of the respiratory chain and the ATP synthase, and various membrane-integral transport proteins,
- (iv) the mitochondrial matrix – containing enzymes of fatty acid oxidation, the citric acid cycle, the mitochondrial DNA together with the mitochondrial transcription and protein synthesis machineries (including mitochondrial 60S ribosomes and mitochondrial tRNAs).

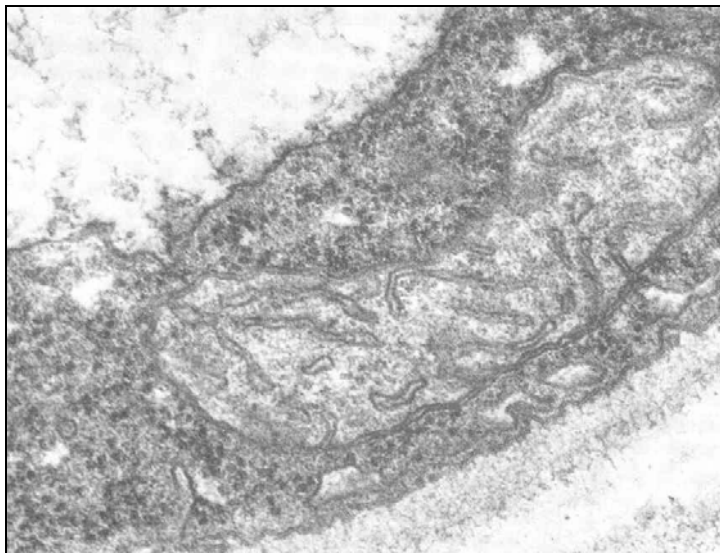


Figure 2-12: Thin section micrograph of a yeast mitochondrion.

Yeast mitochondria are dynamic structures whose size, shape and number can greatly vary according to strain specificity, cell cycle phase, and growth conditions, whereby important factors are: partial oxygen pressure, glucose concentration, presence of unfermentable substrates, availability of sterols and fatty acids, and of particular metal ions (Mg^{++}) (Table 2-5).

Table 2-5: Effects of nutrition on yeast mitochondria.

| Nutrient | Concentration | Oxygen | Respiration | Morphology |
|----------|---------------|--------|-------------|------------|
| Glucose | excess | + | repressed | few large |
| Ethanol | excess | + | activated | many small |
| Glucose | excess | - | repressed | few large |
| Glucose | limited | - | repressed | few large |
| Glucose | limited | + | activated | many small |

Under aerobic conditions, yeast mitochondria are involved in ATP synthesis coupled to oxidative phosphorylation. The activities of the citric acid cycle and the respiratory chain will largely depend on the yeast species and the expression of the Crabtree effect. This is a phenomenon related that relates glucose concentrations with the particular catabolic pathway adopted by glucose-sensitive cells, in that even in the presence of oxygen fermentation predominates over respiration. Under anaerobic conditions, mitochondria seem to be dispensable, at least for respiratory function. In fact, so-called ρ^0 ,petite' mutants that lack functional mitochondria are viable. However, mitochondria do perform other functions in yeast cell physiology, implicating that mitochondria are relevant to intact cell metabolism even under anaerobic conditions:

- synthesis and desaturation of fatty acids and lipids,
- biosynthesis of ergosterol,
- stress responses and adaptation to stresses,
- enzymes for the synthesis of particular amino acids and dicarboxylic acids, pyrimidine and purine bases, porphyrin, and pteridines,
- mobilization of glycogen,
- production of ,flavor' components.

The importance of yeast mitochondria is best illustrated by the fact that some 8 to 10% of the nuclear yeast genes are involved in biogenesis of these organelles and maintenance of their functions. The vast majority of these proteins are synthesized by cytosolic ribosomes and become imported into yeast mitochondria, which have the potential to biosynthesize only 12 different proteins (cytochromoxidase subunits, cytochrome b, the 6 subunits of NADH dehydrogenase, splicing factors) in addition to the mitochondrial rRNAs (15S and 26S subunits) and the complement of mitochondrial tRNAs. The biogenesis of mitochondria, which involves genetic cooperativity between nuclear and mitochondrial genomes, has been widely studied in *S. cerevisiae*, since several kinds of mutations can be used in this model organism. The special features attributed to yeast mitochondria are summarized in Figure 2-13. Details of the genomic structure and content of yeast mitochondrial DNA will be dealt with in chapter 5.

| | |
|-------------------------------|---|
| Mitochondrial genome = 76 Kb | Mitochondrial mutations: |
| Mitochondrial genes: | syn = point mutations in tRNA or rRNA genes |
| rRNA genes | mit = point mutations in respiratory genes |
| tRNA gene complement | Petites (ρ mutants) |
| (except tRNA(Lys imported)) | ρ^0 = no mitochondrial DNA |
| Cytochrome b subunits | ρ^- = segments of mitochondrial DNA |
| Cytochrome-oxidase subunits | repeated to full length |
| ATP-Synthase subunits | |
| Protein-encoding genes | 300 - 400 proteins have to be imported |
| contain introns, which encode | |
| 'maturases' | |

Figure 2-13: Features of yeast mitochondria.

References

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