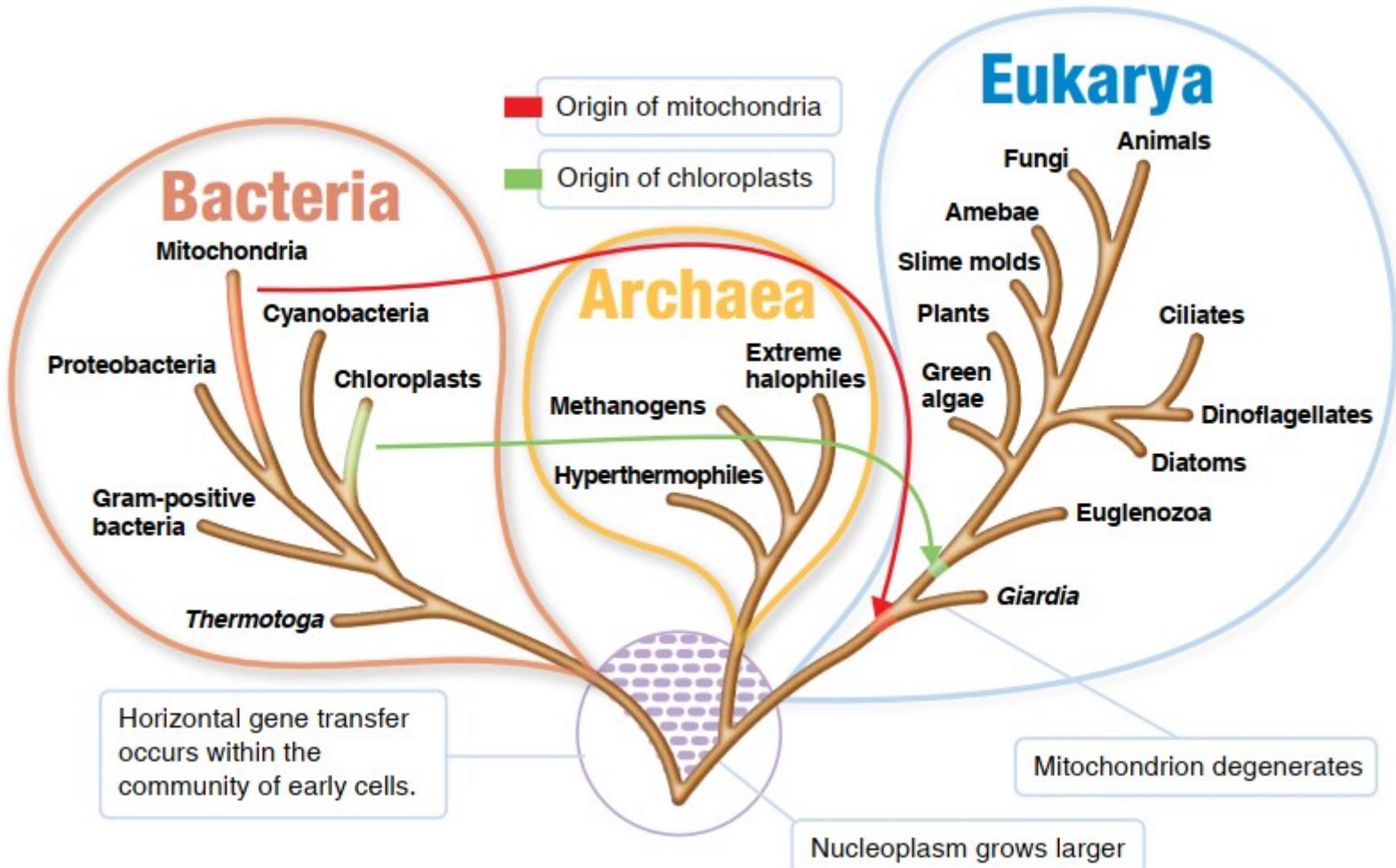


yeasts



Learning outcomes

1. Compare the cellular structures and function of the cellular structures of the yeast cells and bacterial cells.
2. Interpret the yeast reproduction procedures.
3. Classify the yeasts.
4. Compare advantages and disadvantages when enumerating yeast cells by the direct counting method using the red-blood cell counting chamber versus the method of counting colonies on the agar plate.

- Morphology
- Structures and functions
- Reproduction
- Classification
- Quantification

Saccharomyces cerevisiae

Morphology, structures & functions

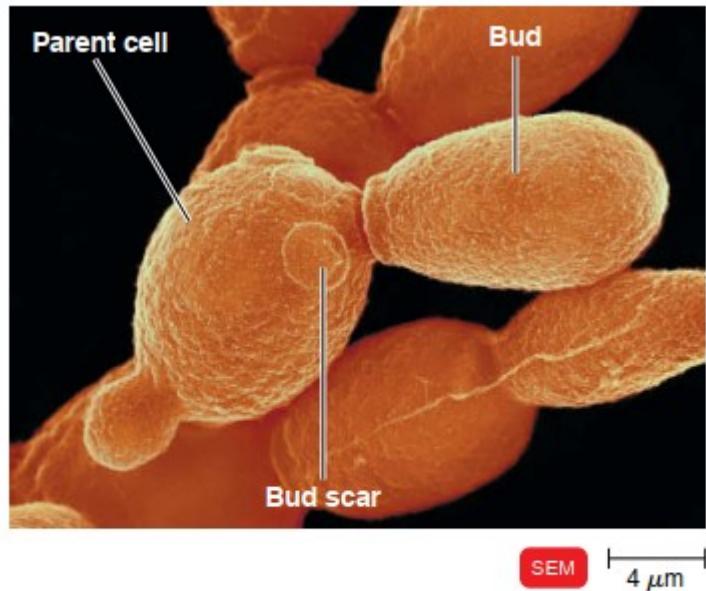
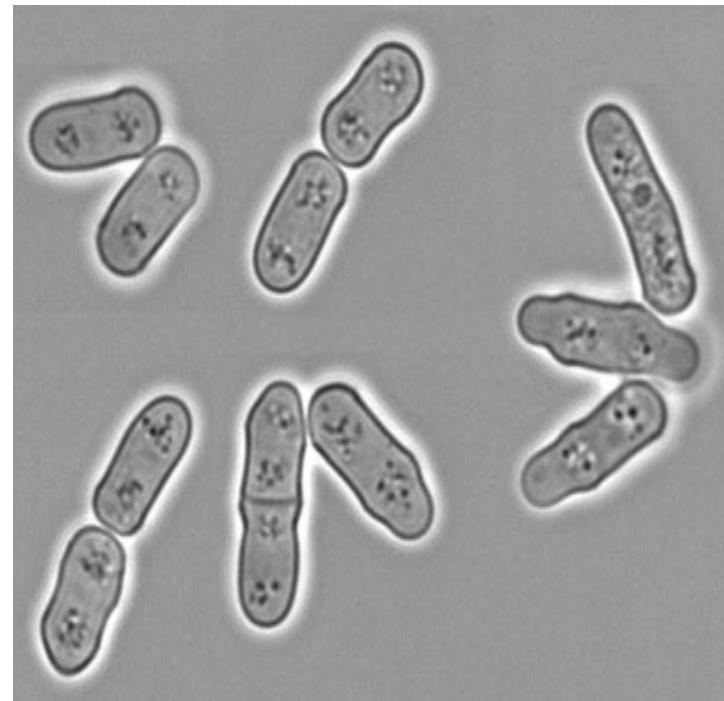


Figure 12.4 A budding yeast. A micrograph of *Saccharomyces cerevisiae* in various stages of budding.

Q How does a bud differ from a spore?



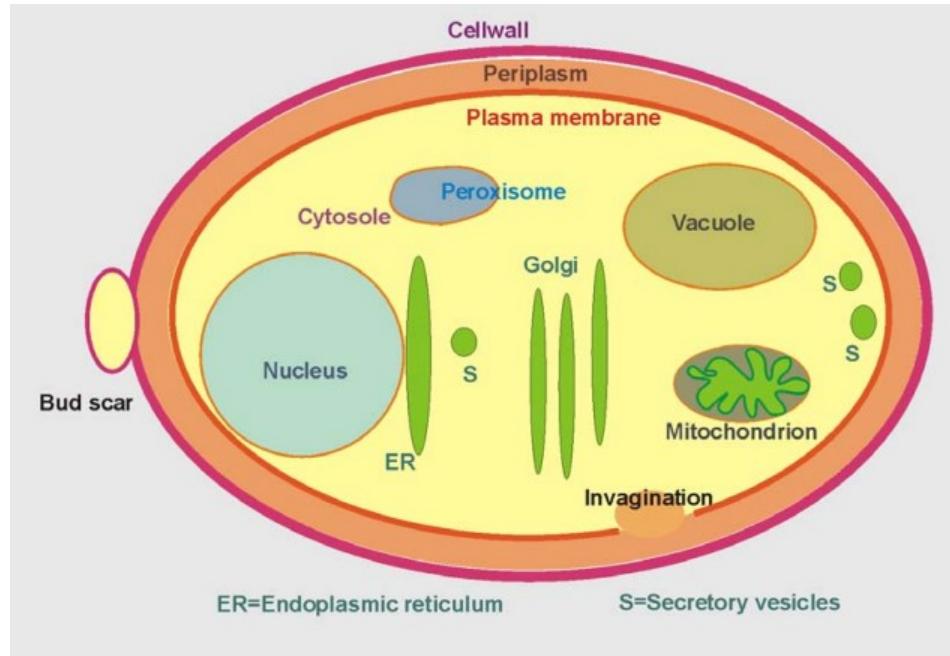
*Saccharomyces
cerevisiae*(budding)



*Schizosaccharomyces
pombe*(fission)

Saccharomyces cerevisiae

Morphology, structures & functions



Cellular structures of *Saccharomyces cerevisiae*.

Saccharomyces cerevisiae

Morphology, structures & functions

□ Cell wall

- ❖ Mainly polysaccharides:
 - ✓ **Glucan** (D-glucose, main chain: β -1,3; branched chain: β -1,6)
 - ✓ **Mannan** (D-glucose, main chain : α -1,6; branched chain : α -1,2 and α -1,3)
 - ✓ **Chitin** (N-acetylglucosamine, β -1,4) mainly in scars.
- ❖ Others :
 - ✓ Mannoproteins
 - ✓ Lipids
 - ✓ Inorganic phosphates

Saccharomyces cerevisiae

Morphology, structures & functions

□ Periplasmic space

- ✓ Mannoprotein
- ✓ Invertase: sucrose => glucose + fructose
- ✓ Phosphatase: inorganic phosphates => inorganic compounds

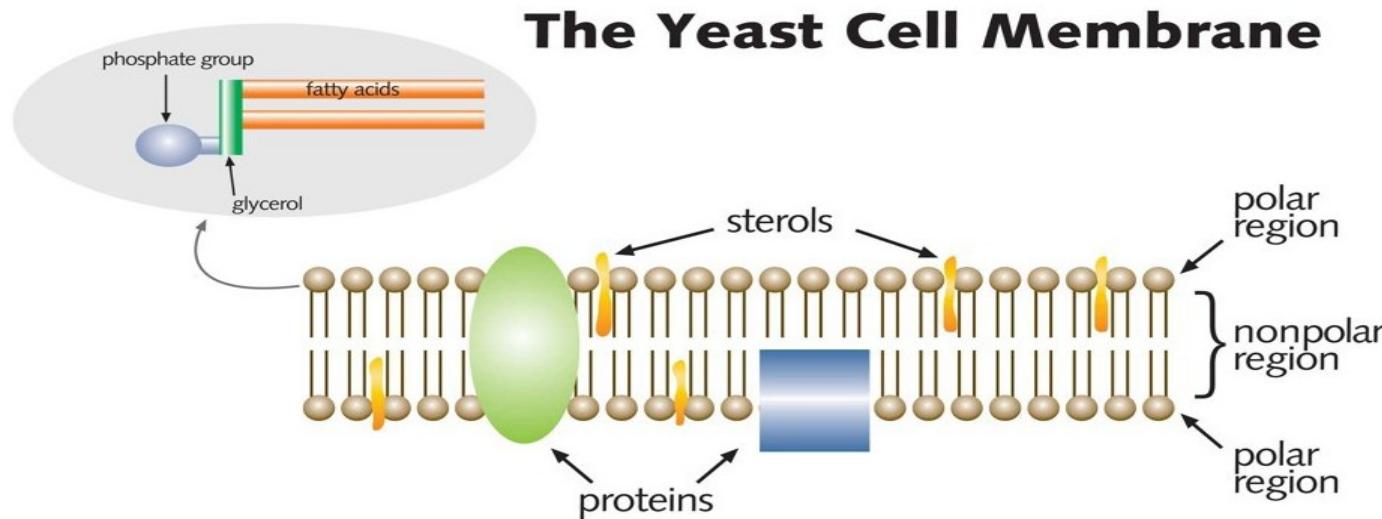
Saccharomyces cerevisiae

Morphology, structures & functions

□ Plasma membrane

- ✓ Phospholipid bilayer
- ✓ Protein

=> semi-permeable (selective).



Saccharomyces cerevisiae

Morphology, structures & functions

□ Cytosol and cytoskeleton

- ✓ Contains ions, organic compounds with low and medium molecular weights, and also contains soluble molecules with large molecular weight such as enzymes, glycogen ...
- ✓ Contains 80S ribosomes, lipid granules and protein complexes

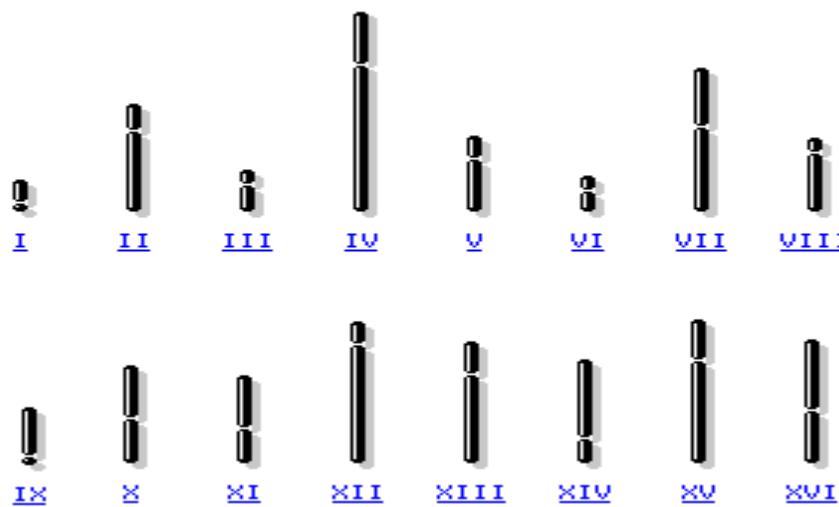
- ✓ The cytoskeleton is made of microtubules and microfilaments, responsible for maintaining cell shape and the stability of the structures inside the cell; the cytoskeleton is also involved in the movement of the organelles inside the cell and in the processes towards the cell division

Saccharomyces cerevisiae

Morphology, structures & functions

❑ Nucleus and extrachromosomal DNA

- ✓ The nucleus is surrounded by a nuclear membrane with nuclear pores.
- ✓ The genome consists of 16 straight chromosomes: DNA + proteins



Saccharomyces cerevisiae

Morphology, structures & functions

❑ Nucleus and extrachromosomal DNA

❑ *Saccharomyces cerevisiae S288C*

Submitter: Saccharomyces Genome Database

Loc	Type	Name	RefSeq	InSDC	Size (Mb)	GC%	Protein	rRNA	tRNA	Other RNA	Gene	Pseudogene
	Chr	I	NC_001133.9	BK006935.2	0.23	39.3	94	-	4	2	101	1
	Chr	II	NC_001134.8	BK006936.2	0.81	38.3	415	-	13	4	432	-
	Chr	III	NC_001135.6	BK006937.2	0.32	38.5	168	-	10	4	184	2
	Chr	IV	NC_001136.10	BK006938.2	1.53	37.9	766	-	28	4	799	1
	Chr	V	NC_001137.3	BK006939.2	0.58	38.5	287	-	20	9	317	1
	Chr	VI	NC_001138.5	BK006940.2	0.27	38.7	128	-	10	4	143	1
	Chr	VII	NC_001139.9	BK006941.2	1.09	38.1	539	-	36	10	585	-
	Chr	VIII	NC_001140.6	BK006934.2	0.56	38.5	290	-	11	4	305	-
	Chr	IX	NC_001141.2	BK006942.2	0.44	38.9	213	-	10	3	232	6
	Chr	X	NC_001142.9	BK006943.2	0.75	38.4	362	-	24	6	392	-
	Chr	XI	NC_001143.9	BK006944.2	0.67	38.1	317	-	16	5	338	-
	Chr	XII	NC_001144.5	BK006945.2	1.08	38.5	519	12	21	18	572	2
	Chr	XIII	NC_001145.3	BK006946.2	0.92	38.2	469	-	21	15	505	-
	Chr	XIV	NC_001146.8	BK006947.3	0.78	38.6	398	-	14	6	418	-
	Chr	XV	NC_001147.6	BK006948.2	1.09	38.2	546	-	20	11	579	2
	Chr	XVI	NC_001148.4	BK006949.2	0.95	38.1	472	-	17	6	497	2
	MT		NC_001224.1	-	0.09	17.1	19	2	24	1	46	-

Saccharomyces cerevisiae

Morphology, structures & functions

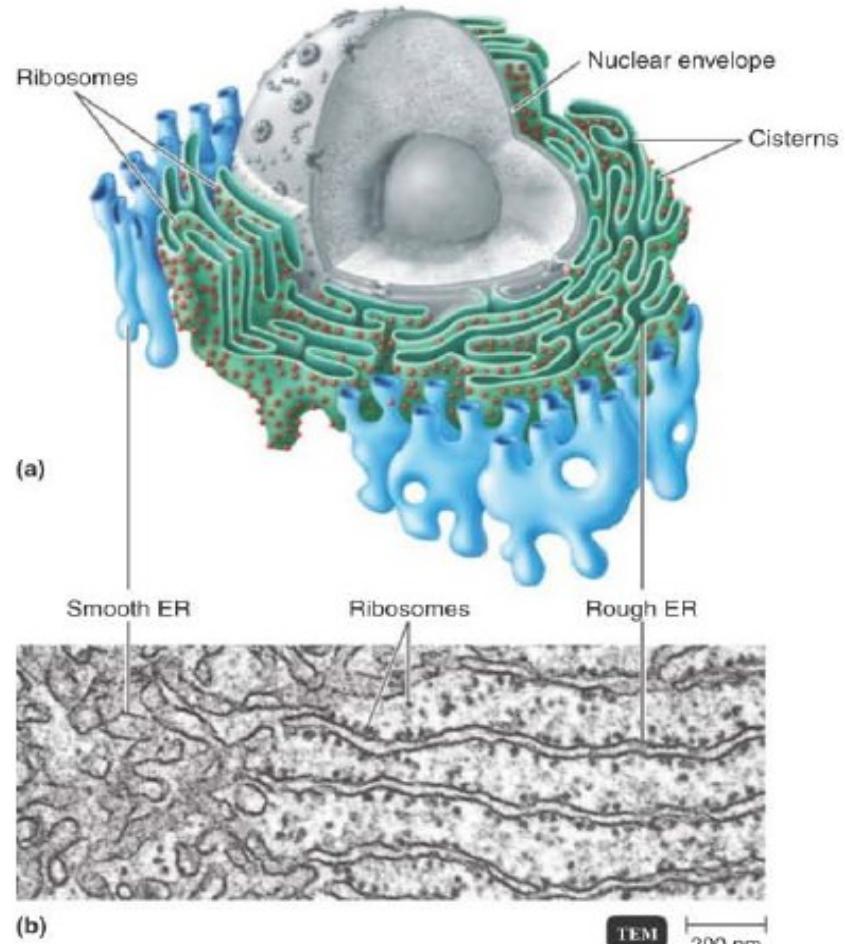
Nucleus and extrachromosomal DNA

- ✓ Mitochondrial DNA and plasmids

Saccharomyces cerevisiae

Morphology, structures & functions

□ Endoplasmic reticulum

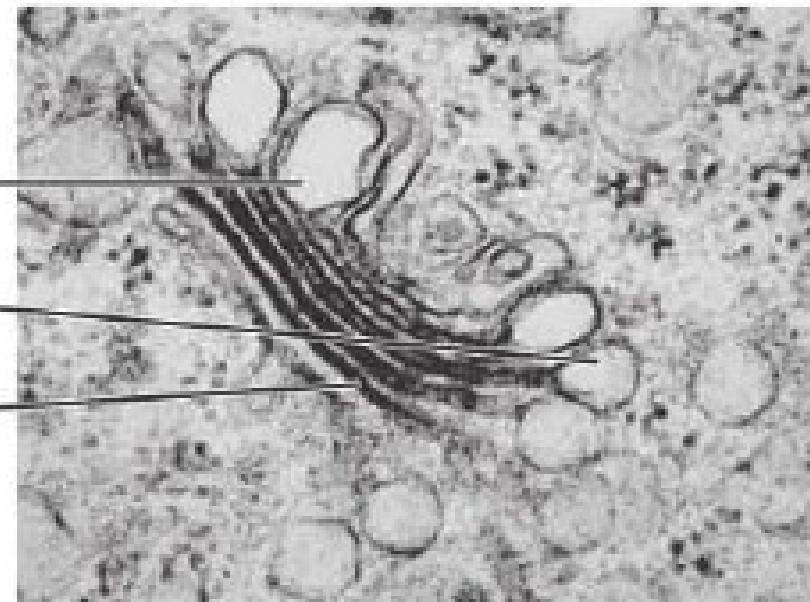
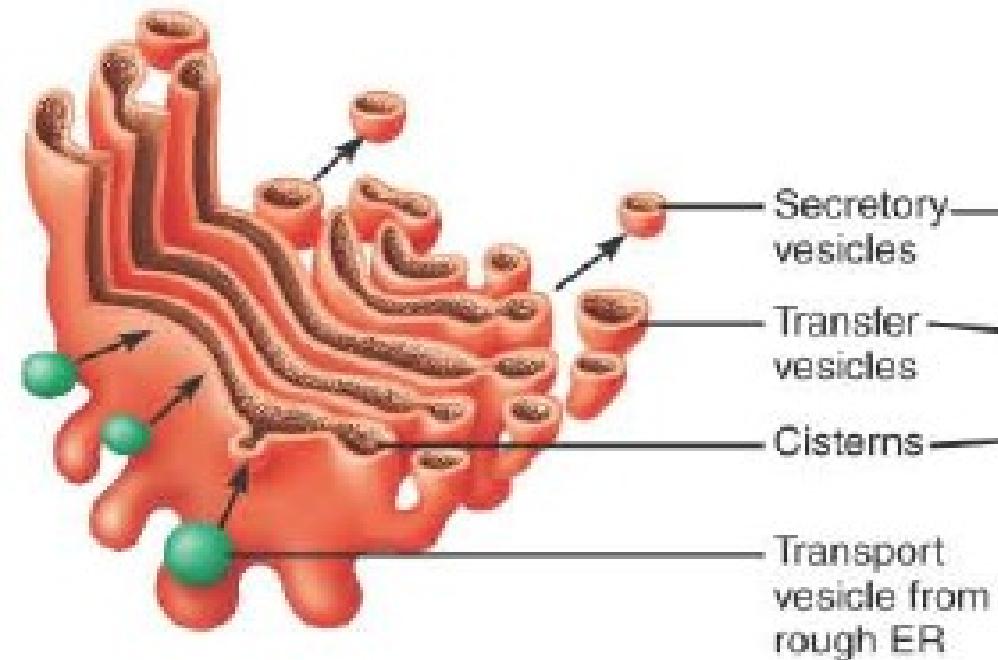


Rough endoplasmic reticulum and ribosomes.
(a) A drawing of details of the endoplasmic reticulum. (b) A micrograph of the endoplasmic reticulum and ribosomes.

Saccharomyces cerevisiae

Morphology, structures & functions

□ Golgi body



TEM

0.25 μm

Saccharomyces cerevisiae

Morphology, structures & functions

□ Vacuole

- ✓ contains enzymes that can hydrolyze the proteins.
- ✓ contains amino acids, polyphosphates, metal ions, which are responsible for regulating the osmotic pressure of the cell.

Saccharomyces cerevisiae

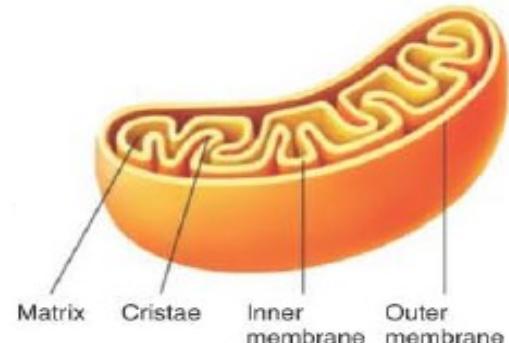
Morphology, structures & functions

□ Peroxisome

- ✓ Peroxisome is an organelle that contains oxidizing enzymes

Saccharomyces cerevisiae

Morphology, structures & functions



Microchondrion

- ✓ Outer membrane: contains enzymes metabolizing lipids
- ✓ Periplasmic space
- ✓ Inner membrane: contains NADH dehydrogenase, succinate dehydrogenase, ATP synthase, integrated transport proteins, ...
- ✓ Mitochondrial matrix: contains enzymes for fatty acid oxidation, the citric acid cycle; contains mitochondrial DNA and components of the mitochondrial DNA replication and transcription.
- ✓ Shape, size and number vary according to species, growth stage and nutritional conditions.

respiration

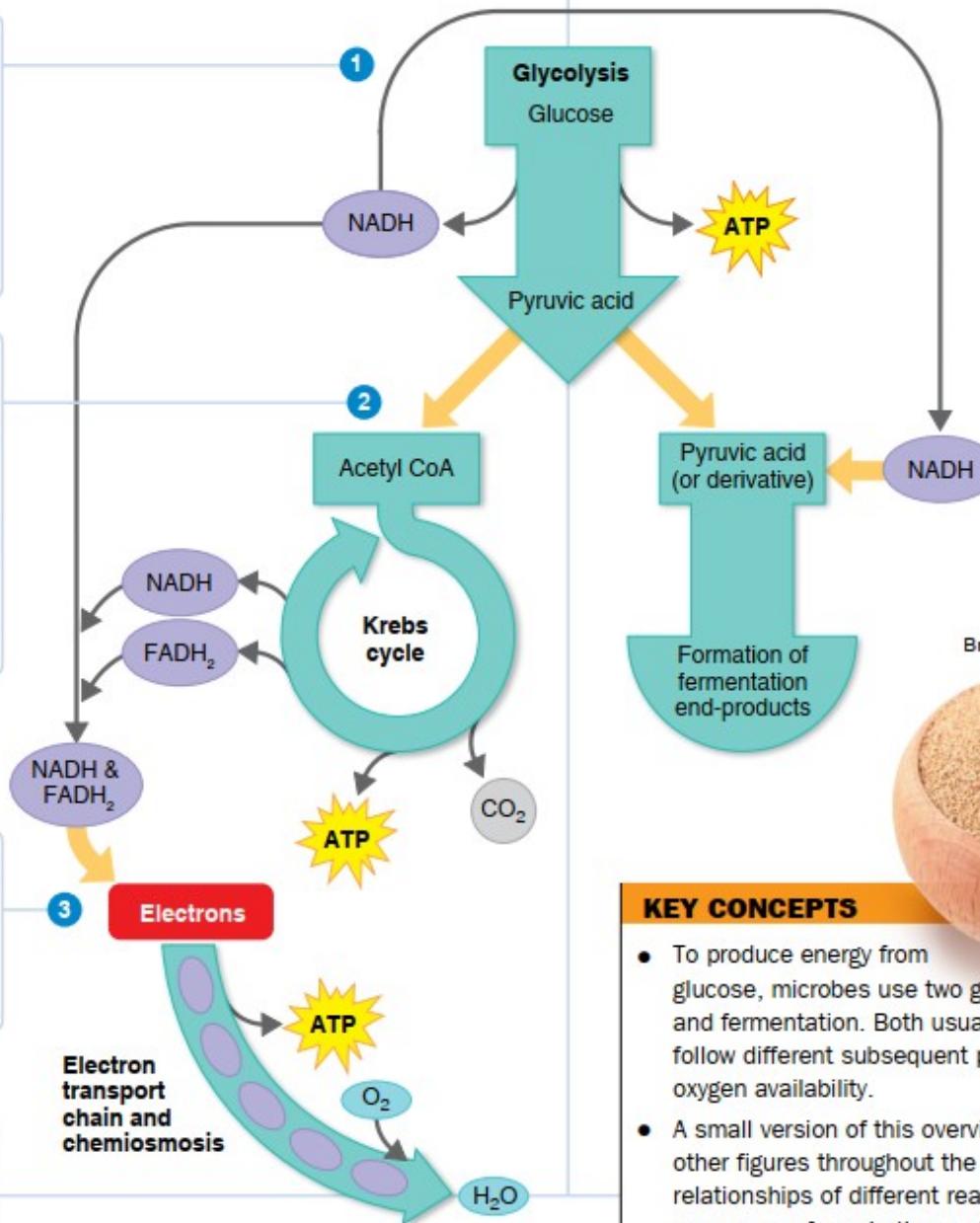
fermentation

Glycolysis produces ATP and reduces NAD⁺ to NADH while oxidizing glucose to pyruvic acid. In respiration, the pyruvic acid is converted to the first reactant in the Krebs cycle, acetyl CoA.

The Krebs cycle produces some ATP by substrate-level phosphorylation, reduces the electron carriers NAD⁺ and FAD, and gives off CO₂. Carriers from both glycolysis and the Krebs cycle donate electrons to the electron transport chain.

In the electron transport chain, the energy of the electrons is used to produce a great deal of ATP by oxidative phosphorylation.

In respiration, the final electron acceptor comes from outside the cell.



In fermentation, the final acceptor is a molecule made in the cell.

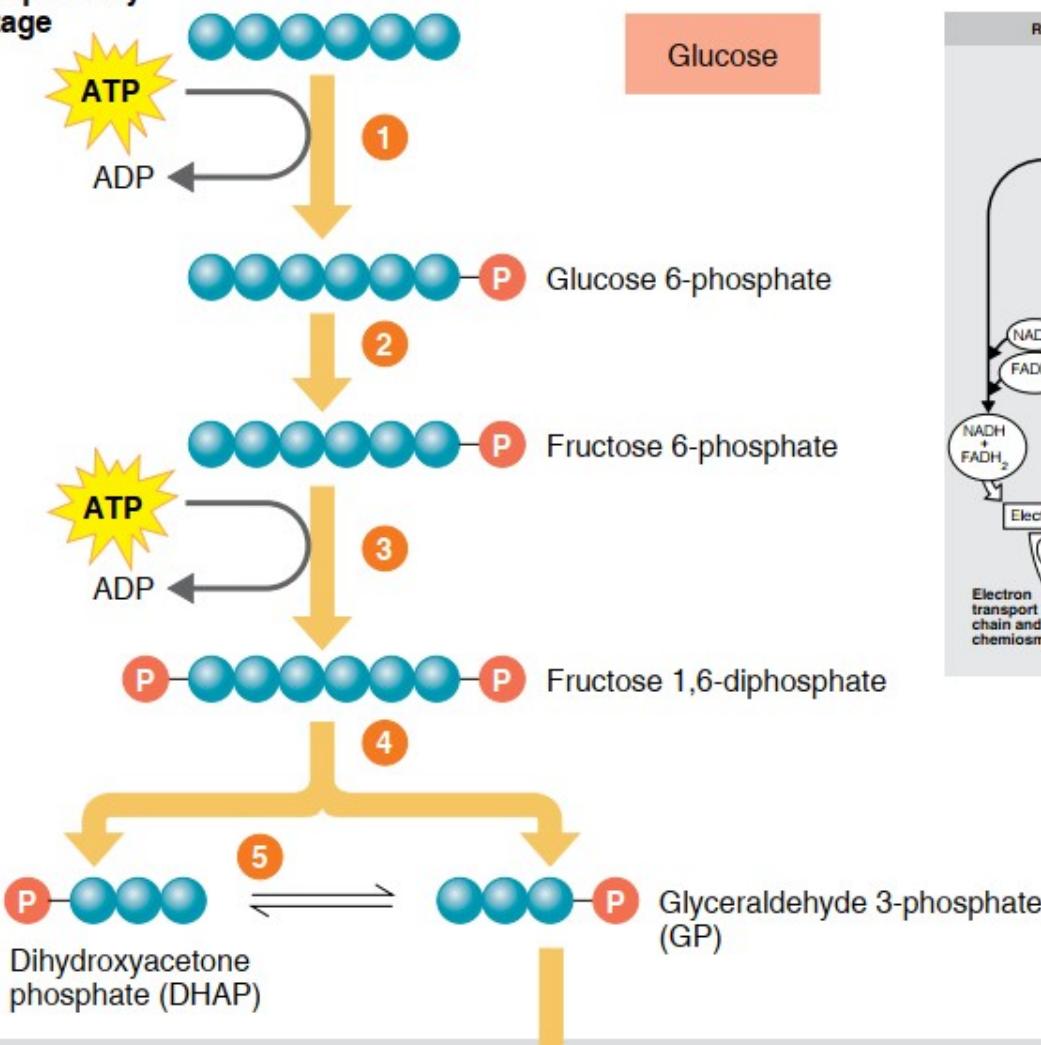
In fermentation, the pyruvic acid and the electrons carried by NADH from glycolysis are incorporated into fermentation end-products.



KEY CONCEPTS

- To produce energy from glucose, microbes use two general processes: respiration and fermentation. Both usually start with glycolysis but follow different subsequent pathways, depending on oxygen availability.
- A small version of this overview figure will be included in other figures throughout the chapter to indicate the relationships of different reactions to the overall processes of respiration and fermentation.

Preparatory stage



Glucose

ATP

ADP

1

2

3

4

5

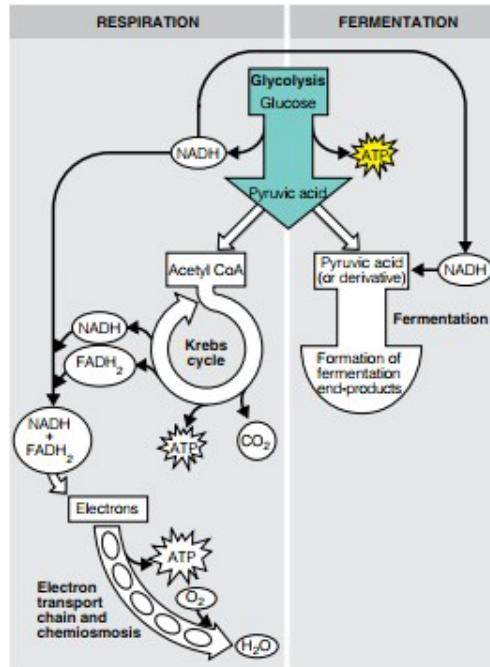
Glucose 6-phosphate

Fructose 6-phosphate

Fructose 1,6-diphosphate

Dihydroxyacetone phosphate (DHAP)

Glyceraldehyde 3-phosphate (GP)



1 Glucose enters the cell and is phosphorylated. A molecule of ATP is invested. The product is glucose 6-phosphate.

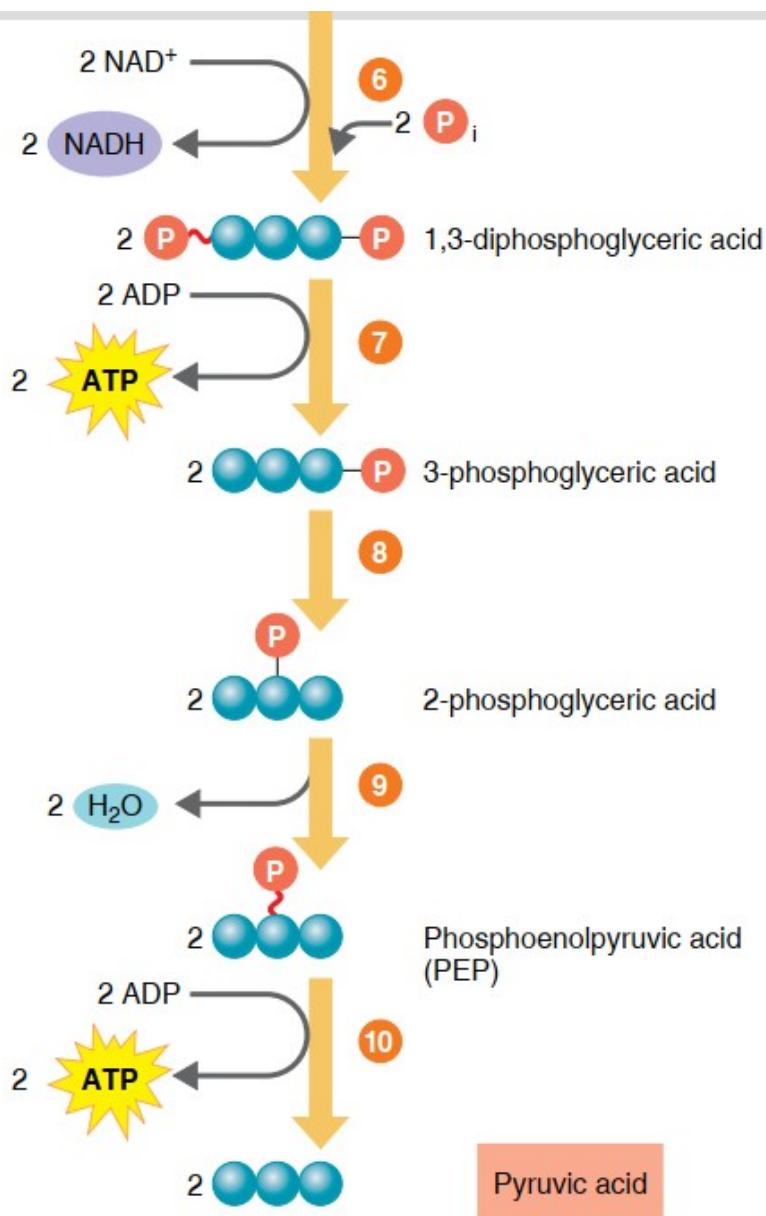
2 Glucose 6-phosphate is rearranged to form fructose 6-phosphate.

3 The P from another ATP is used to produce fructose 1,6-diphosphate, still a six-carbon compound. (Note the total investment of two ATP molecules up to this point.)

4 An enzyme cleaves (splits) the sugar into two three-carbon molecules: dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GP).

5 DHAP is readily converted to GP (the reverse action may also occur).

Energy-conserving stage



6 The next enzyme converts each GP to another three-carbon compound, 1,3-diphosphoglyceric acid. Because each DHAP molecule can be converted to GP and each GP to 1,3-diphosphoglyceric acid, the result is two molecules of 1,3-diphosphoglyceric acid for each initial molecule of glucose. GP is oxidized by the transfer of two hydrogen atoms to NAD^+ to form NADH. The enzyme couples this reaction with the creation of a high-energy bond between the sugar and a P . The three-carbon sugar now has two P groups.

7 The high-energy P is moved to ADP, forming ATP, the first ATP production of glycolysis. (Since the sugar splitting in step 4, all products are doubled. Therefore, this step actually repays the earlier investment of two ATP molecules.)

8 An enzyme relocates the remaining P of 3-phosphoglyceric acid to form 2-phosphoglyceric acid in preparation for the next step.

9 By the loss of a water molecule, 2-phosphoglyceric acid is converted to phosphoenolpyruvic acid (PEP). In the process, the phosphate bond is upgraded to a high-energy bond.

10 This high-energy P is transferred from PEP to ADP, forming ATP. For each initial glucose molecule, the result of this step is two molecules of ATP and two molecules of a three-carbon compound called pyruvic acid.

Figure 5.12 An outline of the reactions of glycolysis (Embden-Meyerhof pathway). The inset indicates the relationship of glycolysis to the overall processes of respiration and fermentation. A more detailed version of glycolysis is presented in Figure A.2 in Appendix A.

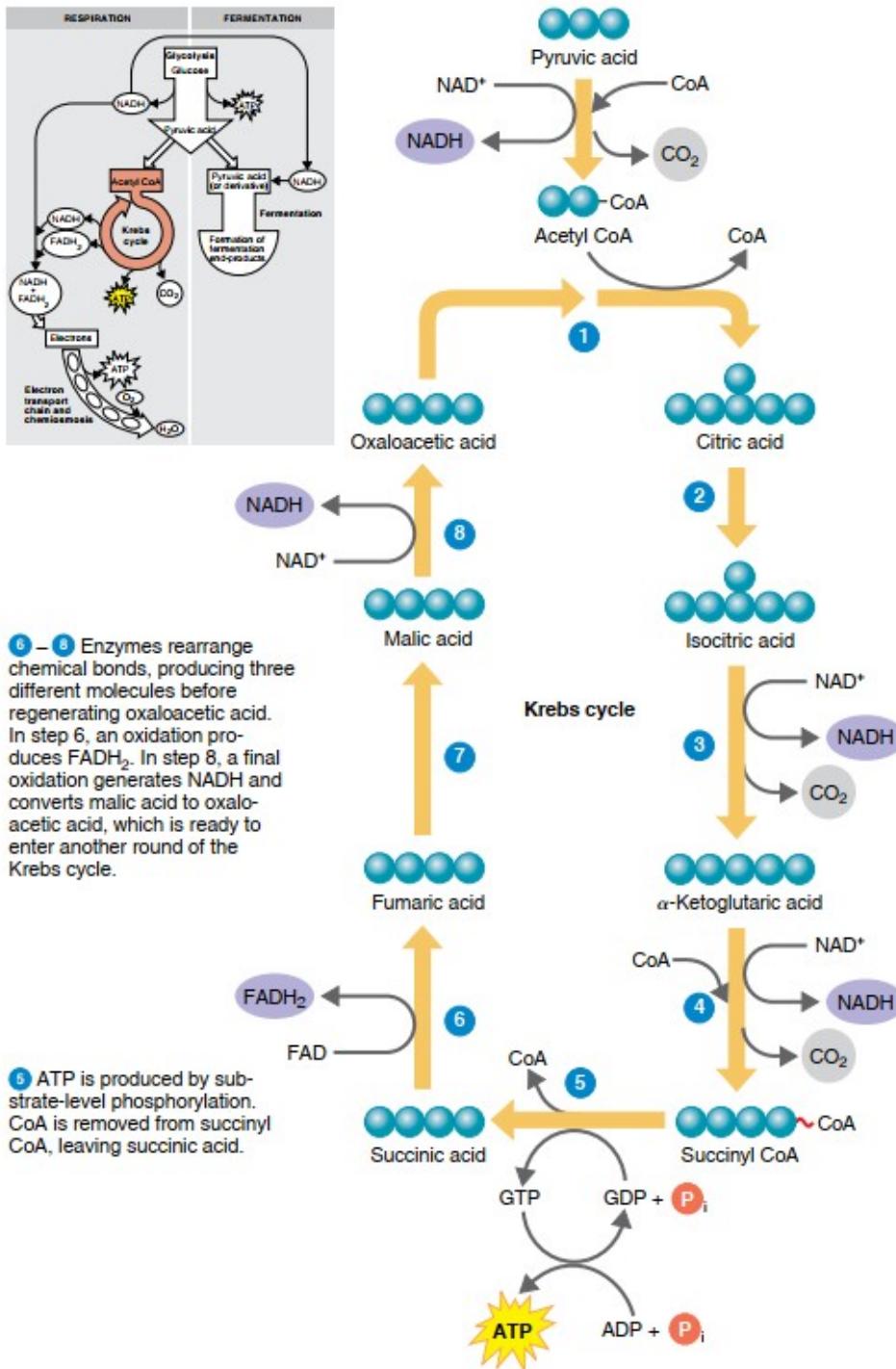


Figure 5.13 The Krebs cycle. The inset indicates the relationship of the Krebs cycle to the overall process of respiration. A more detailed version of the Krebs cycle is presented in Figure A.5 in Appendix A.

Q What are the products of the Krebs cycle?

1 A turn of the cycle begins as enzymes strip off the CoA portion from acetyl CoA and combine the remaining two-carbon acetyl group with oxaloacetic acid. Adding the acetyl group produces the six-carbon molecule citric acid.

2 – 4 Oxidations generate NADH. Step 2 is a rearrangement. Steps 3 and 4 combine oxidations and decarboxylations to dispose of two carbon atoms that came from oxaloacetic acid. The carbons are released as CO₂, and the oxidations generate NADH from NAD⁺. During the second oxidation (step 4), CoA is added into the cycle, forming the compound succinyl CoA.

The Citric Acid Cycle Completes the Energy-yielding Oxidation of Organic Molecules

- Every two CH₃-CO-CoA molecules participating the cycle produce
 - 4 CO₂,
 - 6 NADH,
 - 2 FADH₂ and
 - 2 ATP molecules.

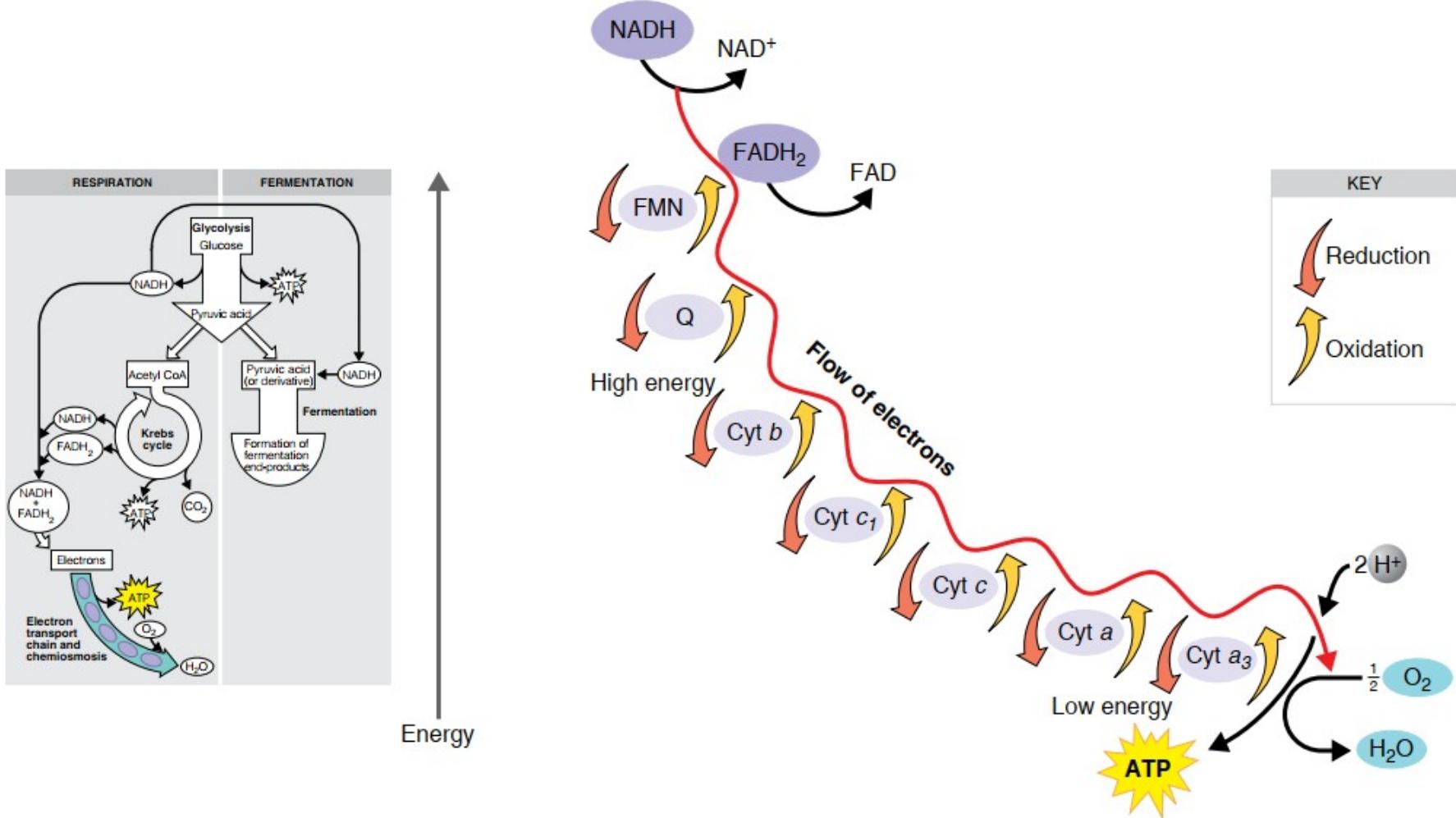


Figure 5.14 An electron transport chain (system). The inset indicates the relationship of the electron transport chain to the overall process of respiration. In the mitochondrial electron transport chain shown, the electrons pass along the chain in a gradual and stepwise fashion, so energy is released in manageable quantities (see Figure 5.16 to learn where ATP is formed).

Q How many ATPs can be made from the oxidation of one NADH in the electron transport chain?

A

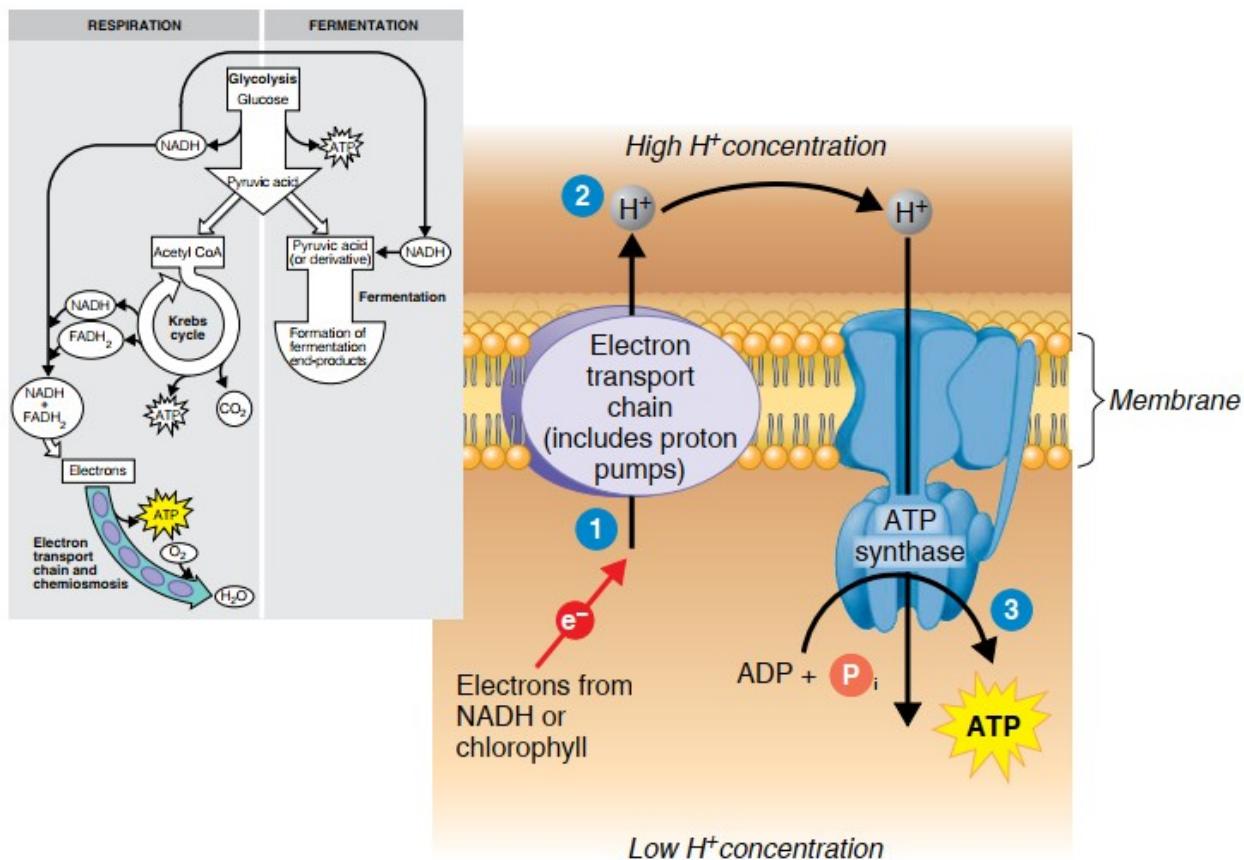


Figure 5.15 Chemiosmosis. An overview of the mechanism of chemiosmosis. The membrane shown could be a prokaryotic plasma membrane, a eukaryotic mitochondrial membrane, or a photosynthetic thylakoid. The numbered steps are described in the text.

Q What is the proton motive force?

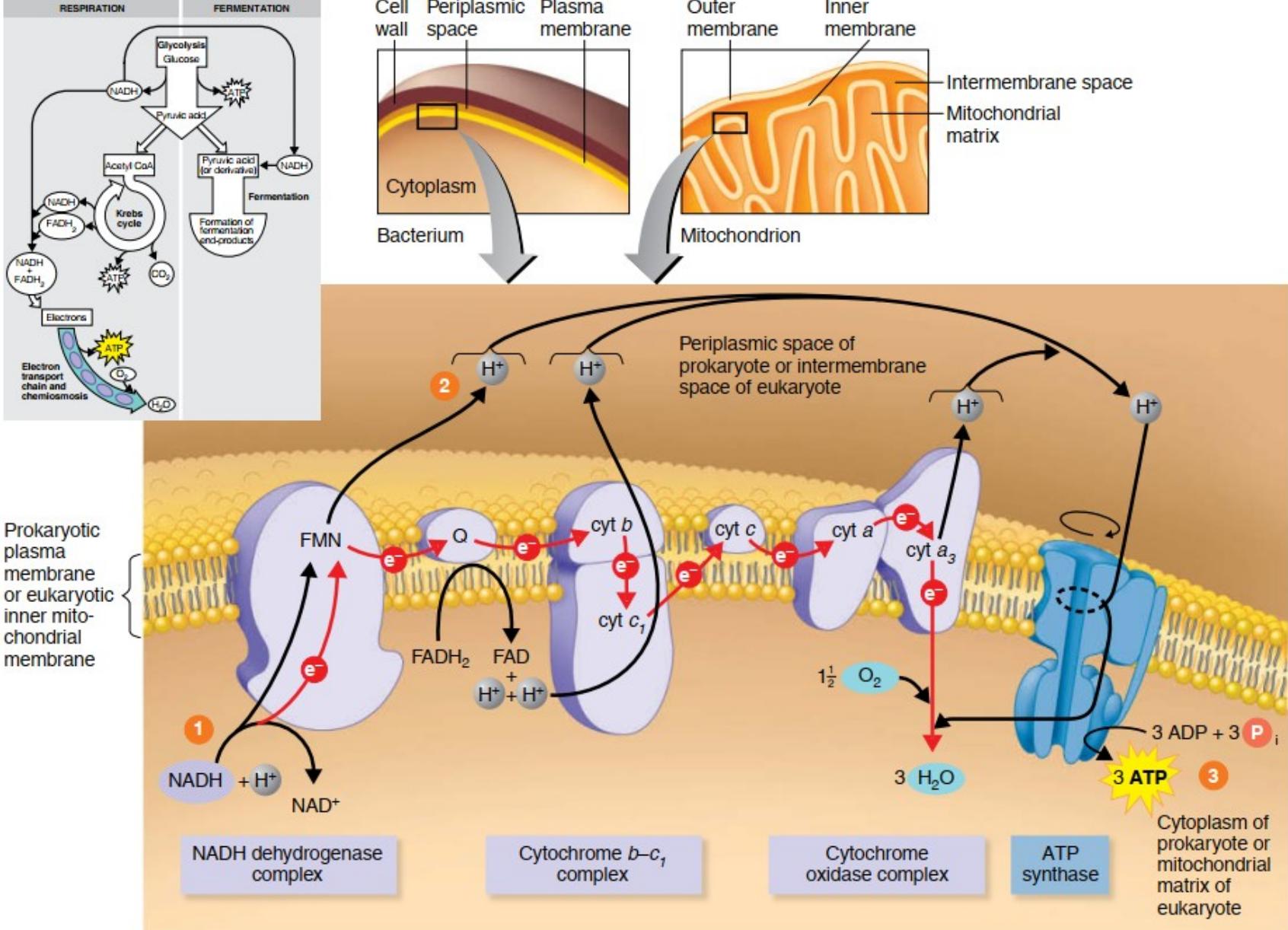


Figure 5.16 Electron transport and the chemiosmotic generation of ATP. Electron carriers are organized into three complexes, and protons (H^+) are pumped across the membrane at three points. In a prokaryotic

cell, protons are pumped across the plasma membrane from the cytoplasmic side. In a eukaryotic cell, they are pumped from the matrix side of the mitochondrial membrane

to the opposite side. The flow of electrons is indicated with red arrows.

Q Where does chemiosmosis occur in eukaryotes? In prokaryotes?

Saccharomyces cerevisiae
is a facultative anaerobe.

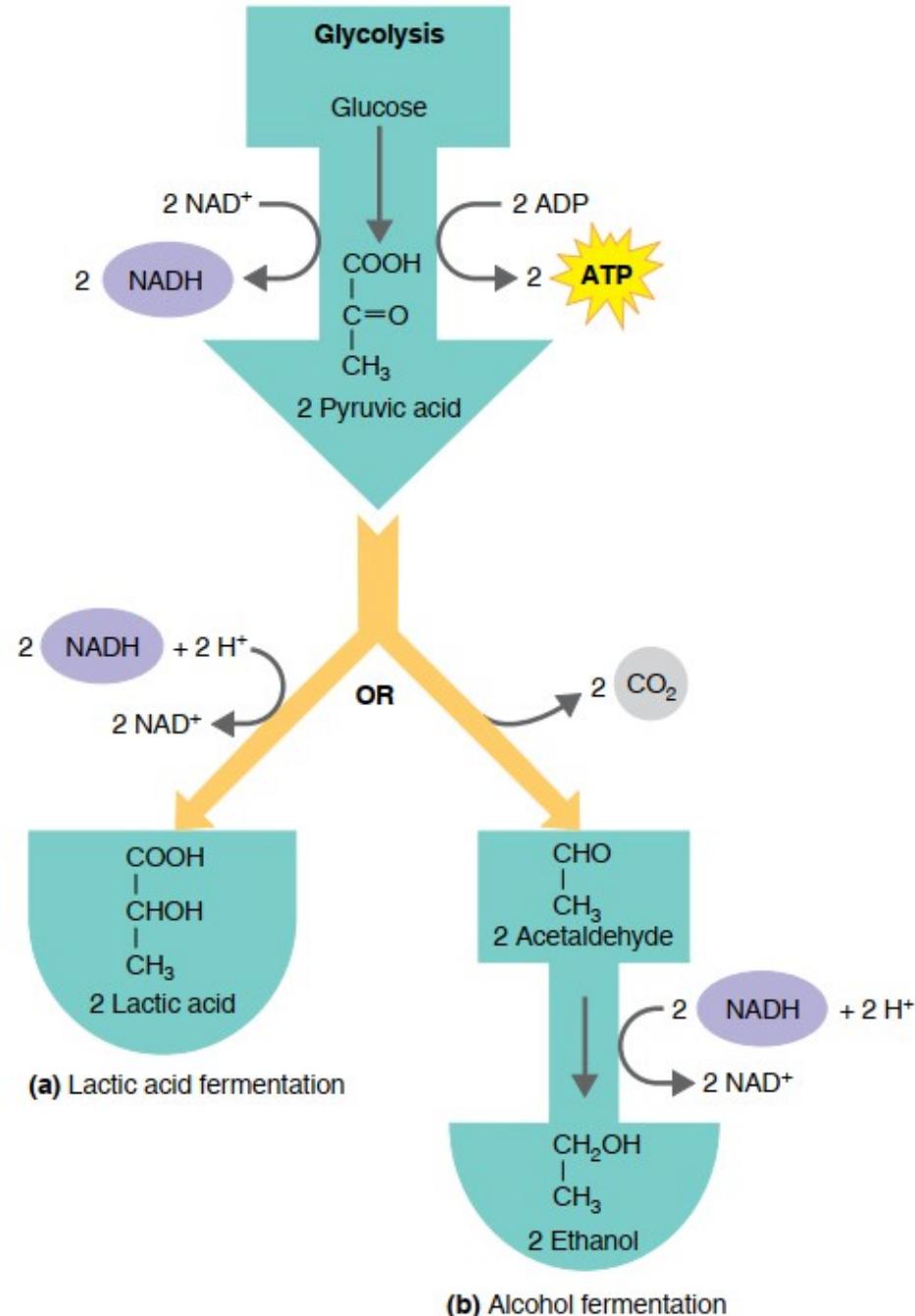
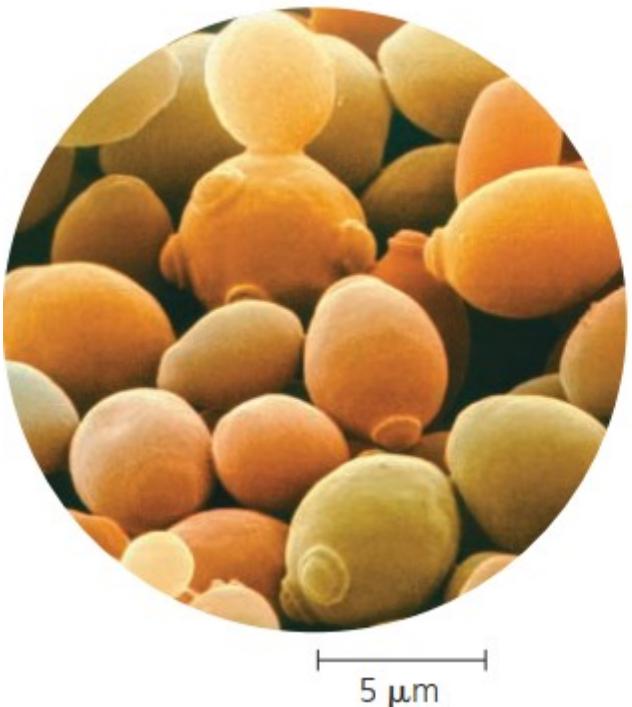


Figure 5.19 Types of fermentation.



Toxic Wastes

Yeasts, such as the brewer's yeast *Saccharomyces cerevisiae*, are used to convert carbohydrates to ethanol in winemaking. The ethanol that accumulates in the wine is toxic to yeasts and contributes to density-dependent regulation of yeast population size. The alcohol content of wine is usually less than 13% because that is the maximum concentration of ethanol that most wine-producing yeast cells can tolerate.

Saccharomyces cerevisiae

Reproduction

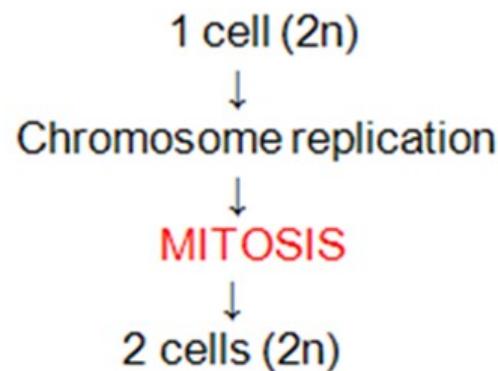
Asexual and sexual reproduction

Saccharomyces cerevisiae

Reproduction

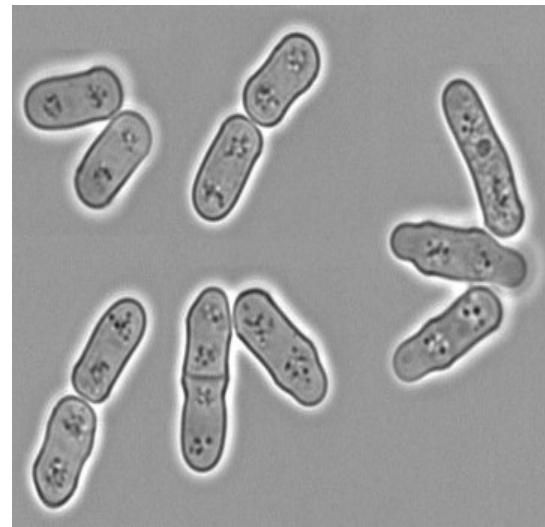
In mitotic division:

- Haploid and diploid cells: Mitosis (budding).





Saccharomyces cerevisiae
(budding)



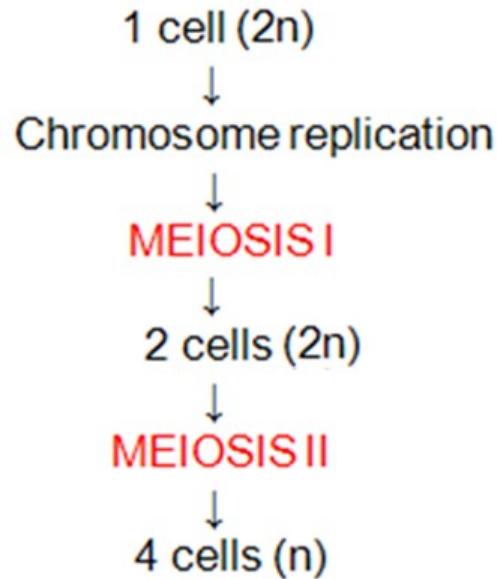
Schizosaccharomyces pombe (fission)

Saccharomyces cerevisiae

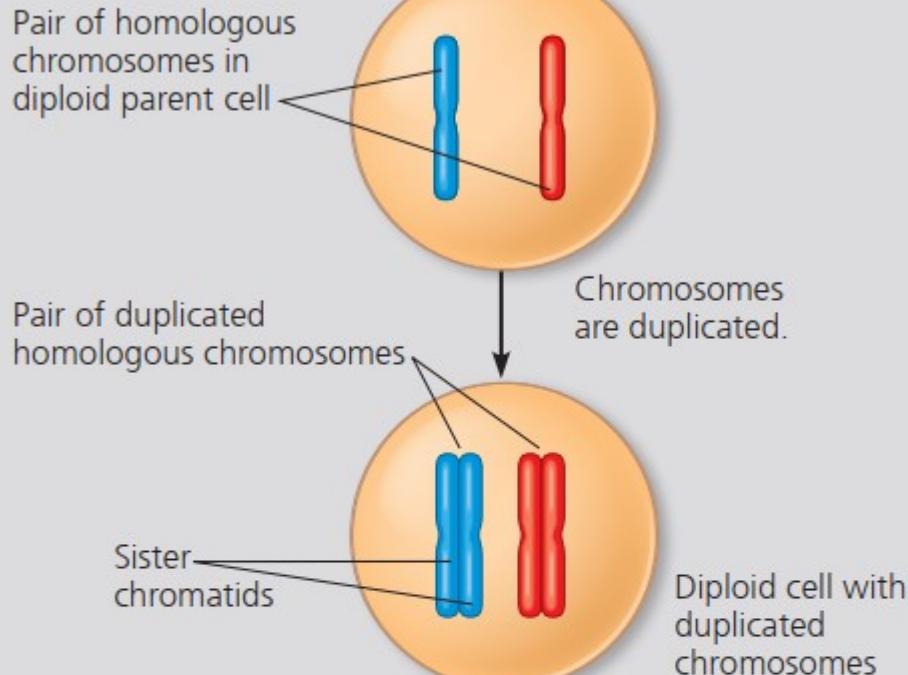
Reproduction

In meiotic division:

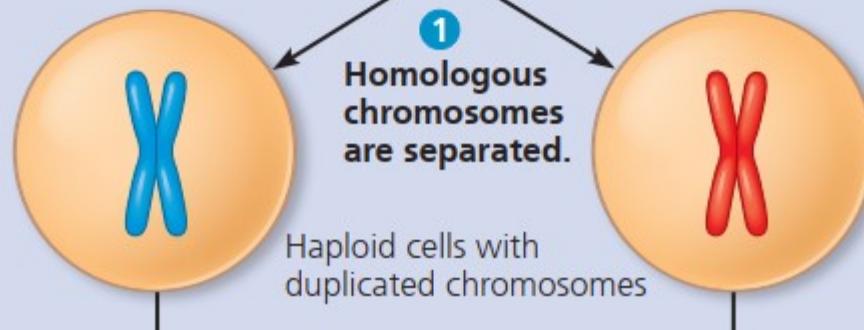
- Mating: Haploid α + haploid $a \Rightarrow$ zygote:
meiosis
 \Rightarrow haploid spores



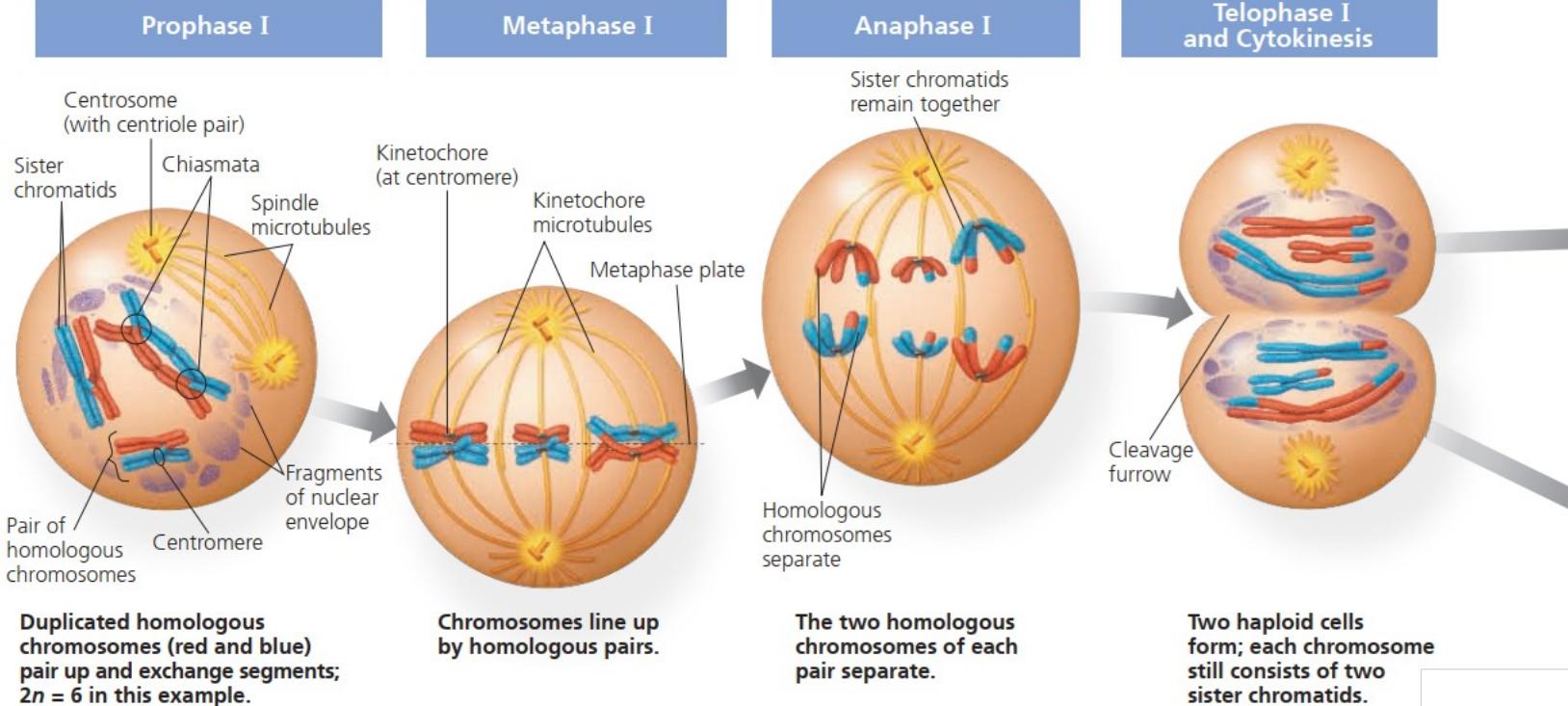
Interphase

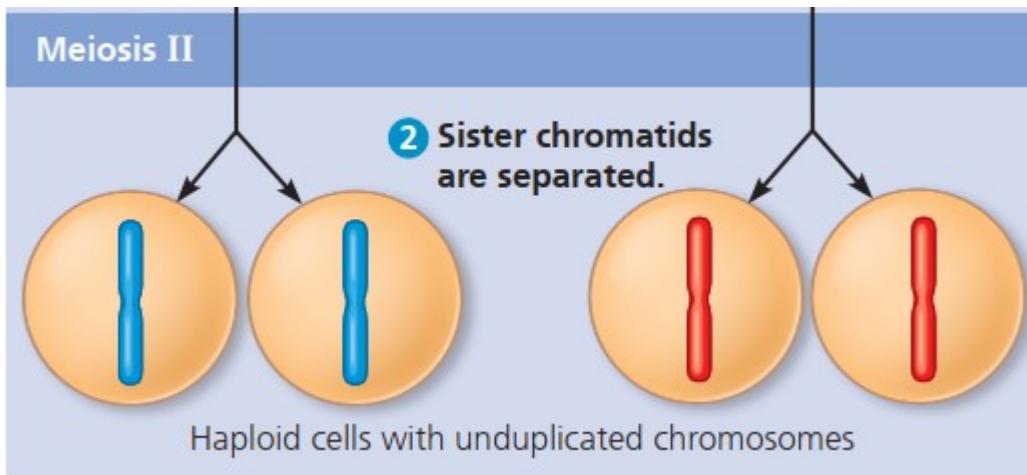


Meiosis I



MEIOSIS I: Separates homologous chromosomes





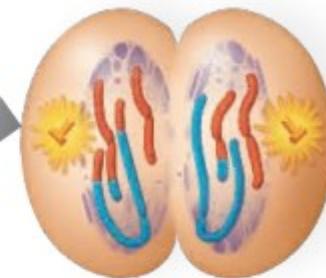
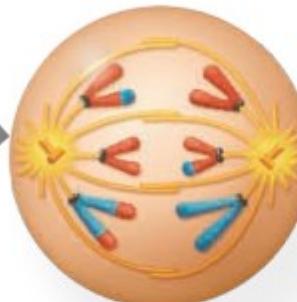
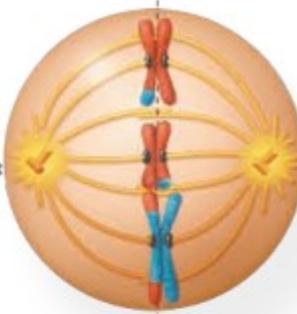
MEIOSIS II: Separates sister chromatids

Prophase II

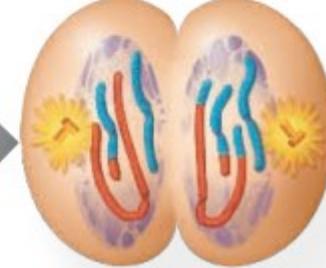
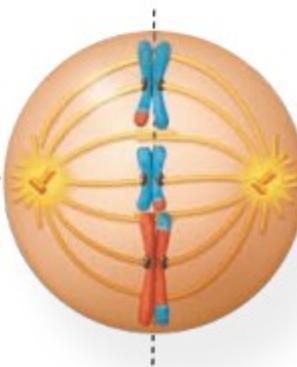
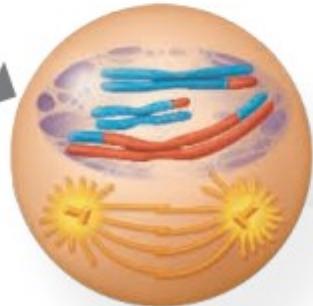
Metaphase II

Anaphase II

Telophase II
and Cytokinesis



During another round of cell division, the sister chromatids finally separate; four haploid daughter cells result, containing unduplicated chromosomes.



Saccharomyces cerevisiae Reproduction

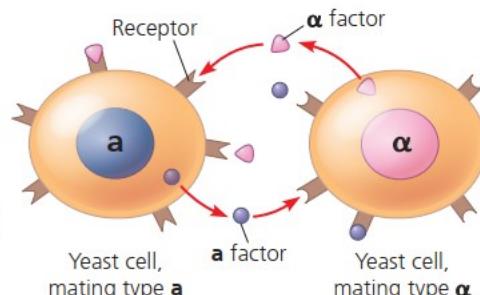
- Mating: Haploid α + haploid $a \Rightarrow$ zygote: meiosis
 \Rightarrow haploid spores

▼ Figure 11.3 Communication between mating yeast cells.

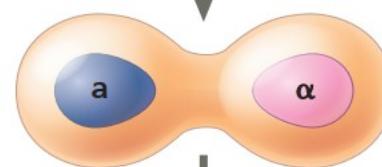
Saccharomyces cerevisiae cells use chemical signaling to identify cells of the opposite mating type and initiate the mating process. The two mating types and their corresponding chemical signaling molecules, or mating factors, are called a and α .

1 Exchange of mating factors.

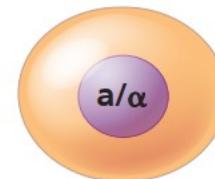
Each mating cell type secretes a mating factor that binds to receptors on the other mating type.



2 Mating. Binding of the factors to receptors induces changes in the cells that lead to their fusion.

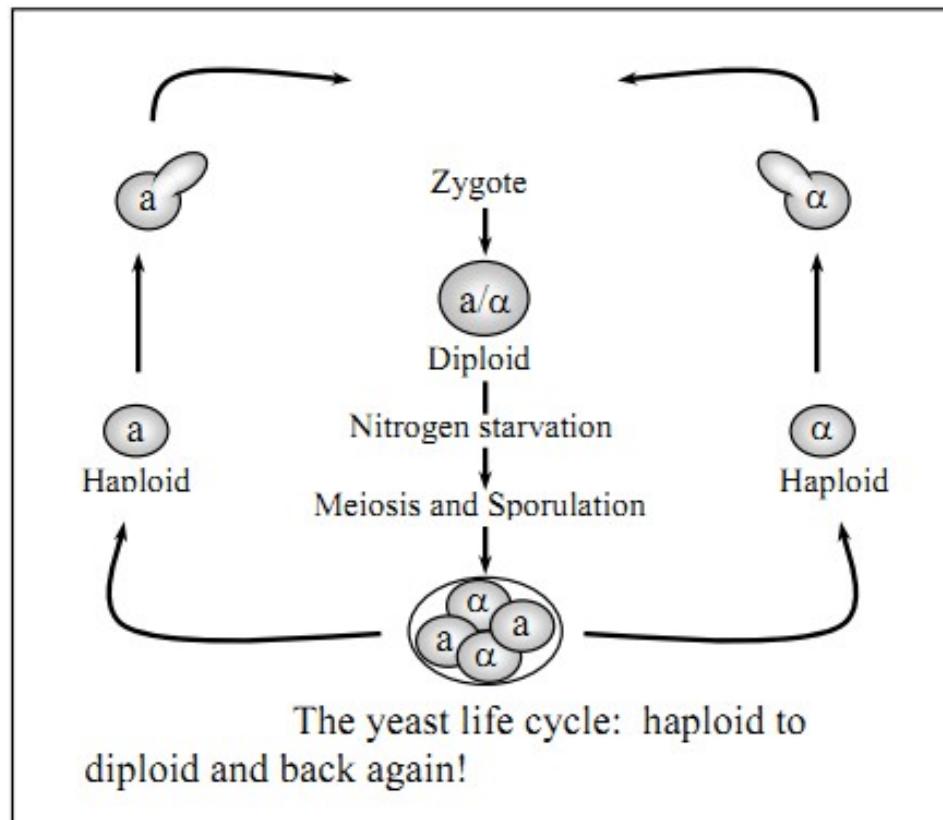


3 New a/α cell. The nucleus of the fused cell includes all the genes from the a and α cells.



Saccharomyces cerevisiae Reproduction

- Mating: Haploid α + haploid $a \Rightarrow$ zygote: meiosis
 \Rightarrow haploid spores

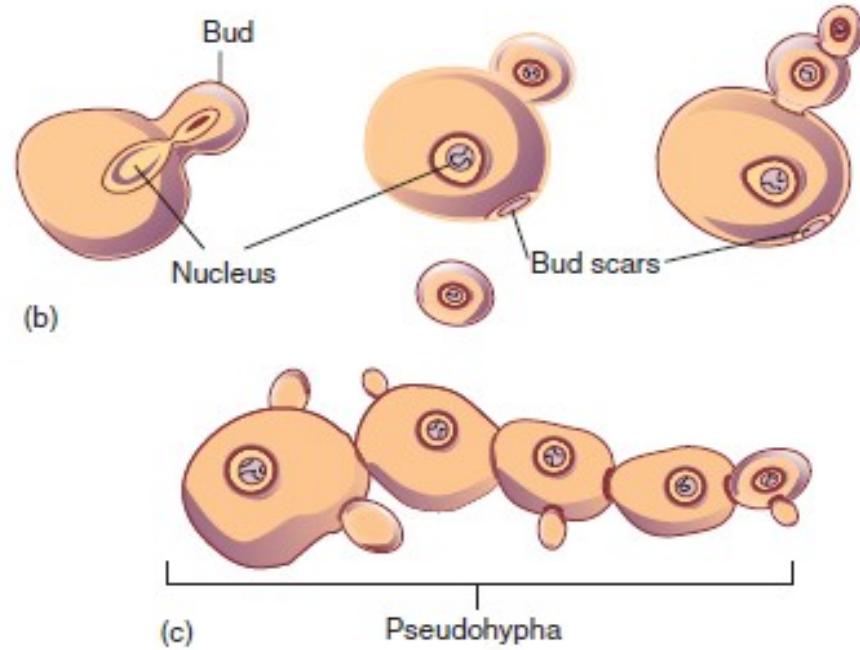


Saccharomyces cerevisiae

Reproduction



(a)



Microscopic morphology of yeasts. (a) Scanning electron micrograph of the brewer's, or baker's, yeast *Saccharomyces cerevisiae* ($21,000\times$). (b) Formation and release of yeast buds. (c) Formation of pseudohypha (a chain of budding yeast cells).

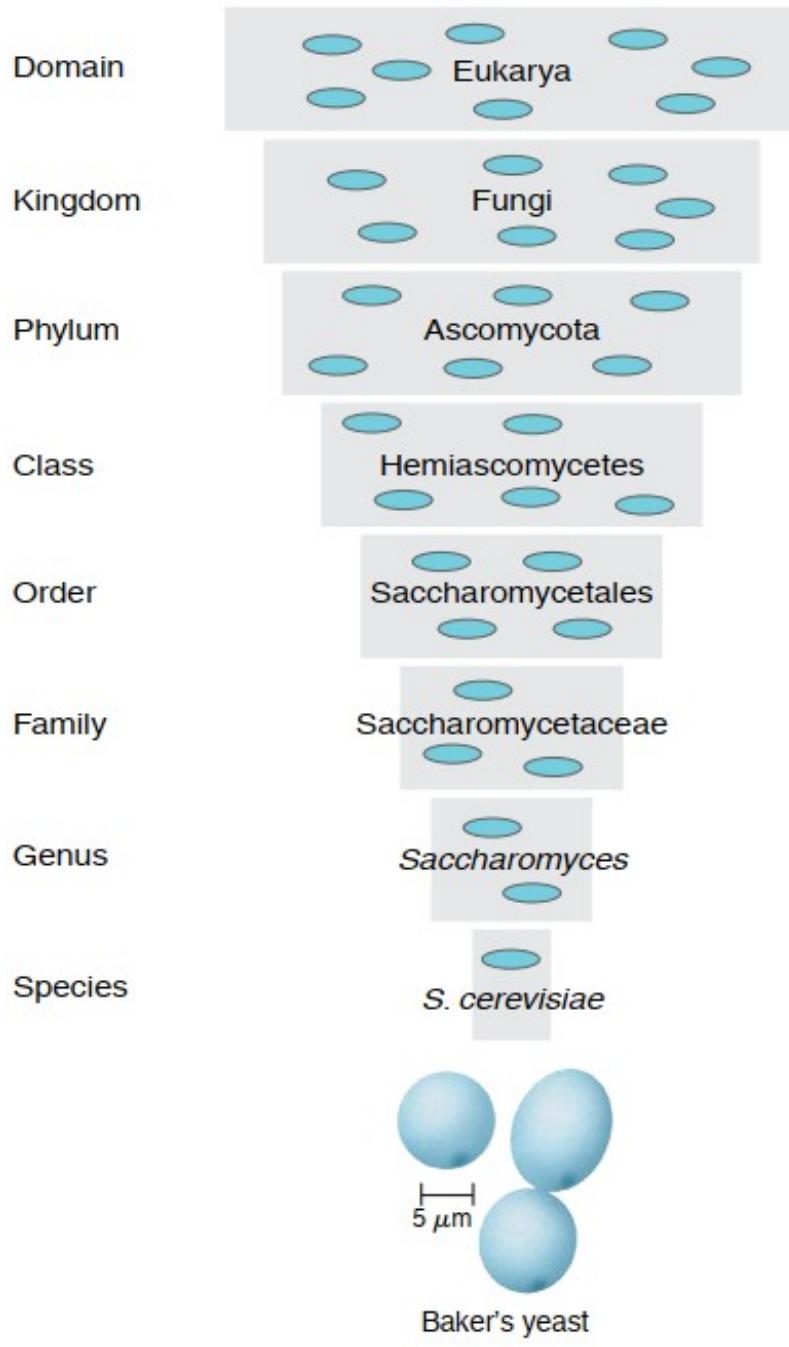
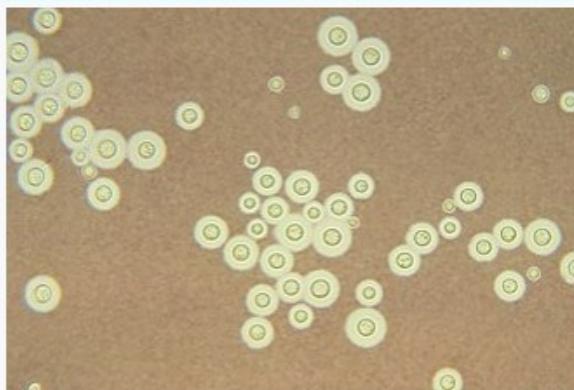


Figure 10.5 The taxonomic hierarchy.

Cryptococcus neoformans



Yeast state of *Cryptococcus
neoformans*

Scientific classification



Kingdom: Fungi
Division: Basidiomycota
Class: Tremellomycetes
Order: Tremellales
Family: Cryptococcaceae
Genus: *Cryptococcus*
Species: *C. neoformans*

Binomial name

Cryptococcus neoformans

(San Felice) Vuill. (1901)

Scientific classification

Domain: Eukaryota

Kingdom: Fungi

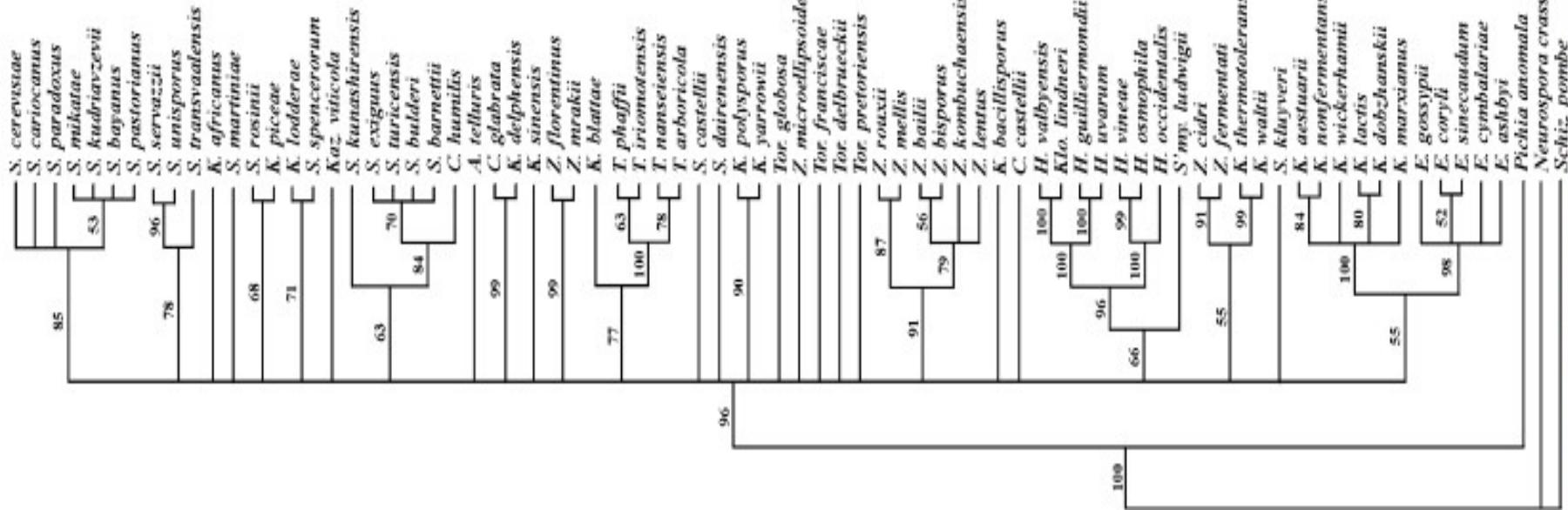
Phyla and Subphyla

Ascomycota p. p.

- Saccharomycotina (true yeasts)
- Taphrinomycotina p. p.
 - Schizosaccharomycetes
(fission yeasts)

Basidiomycota p. p.

- Agaricomycotina p. p.
 - Tremellomycetes
- Pucciniomycotina p. p.
 - Microbotryomycetes



Schizosaccharomyces pombe is the outgroup species. Abbreviations for figures:

A. = *Arxiozyma*, C. = *Candida*, E. = *Eremothecium*, H. = *Hanseniaspora*, K. = *Kluyveromyces*, Kaz. = *Kazachstania*, Klo. = *Kloeckera*, S. = *Saccharomyces*, Schiz. = *Schizosaccharomyces*, S'my. = *Saccharomycodes*, T. = *Tetrapisispora*, Tor. = *Torulaspora*, Z. = *Zygosaccharomyces*.

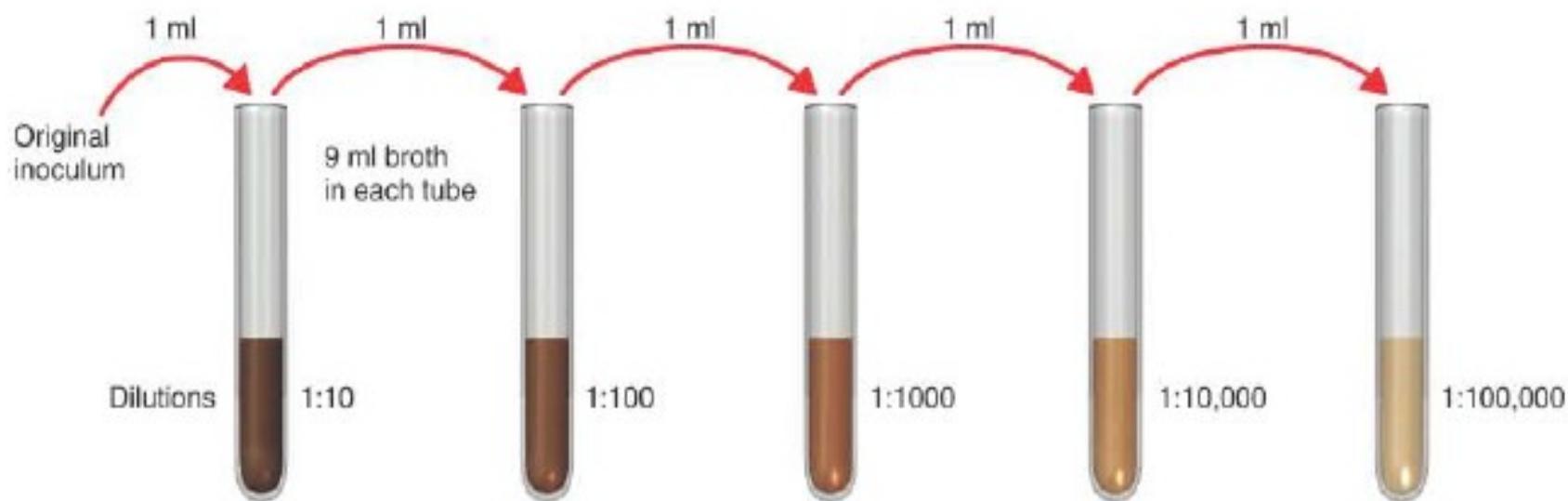
C.P. Kurtzman, C.J. Robnett / FEMS Yeast Research 3 (2003) 417–432

Fig. 3. Bootstrap consensus tree of species in the 'Saccharomyces complex' from MP analysis of 18S rDNA. Bootstrap values >50% are given. Tree length = 1018, CI = 0.509, RI = 0.739, RC = 0.376, S. pombe is the outgroup species, and all species are represented by type strains.

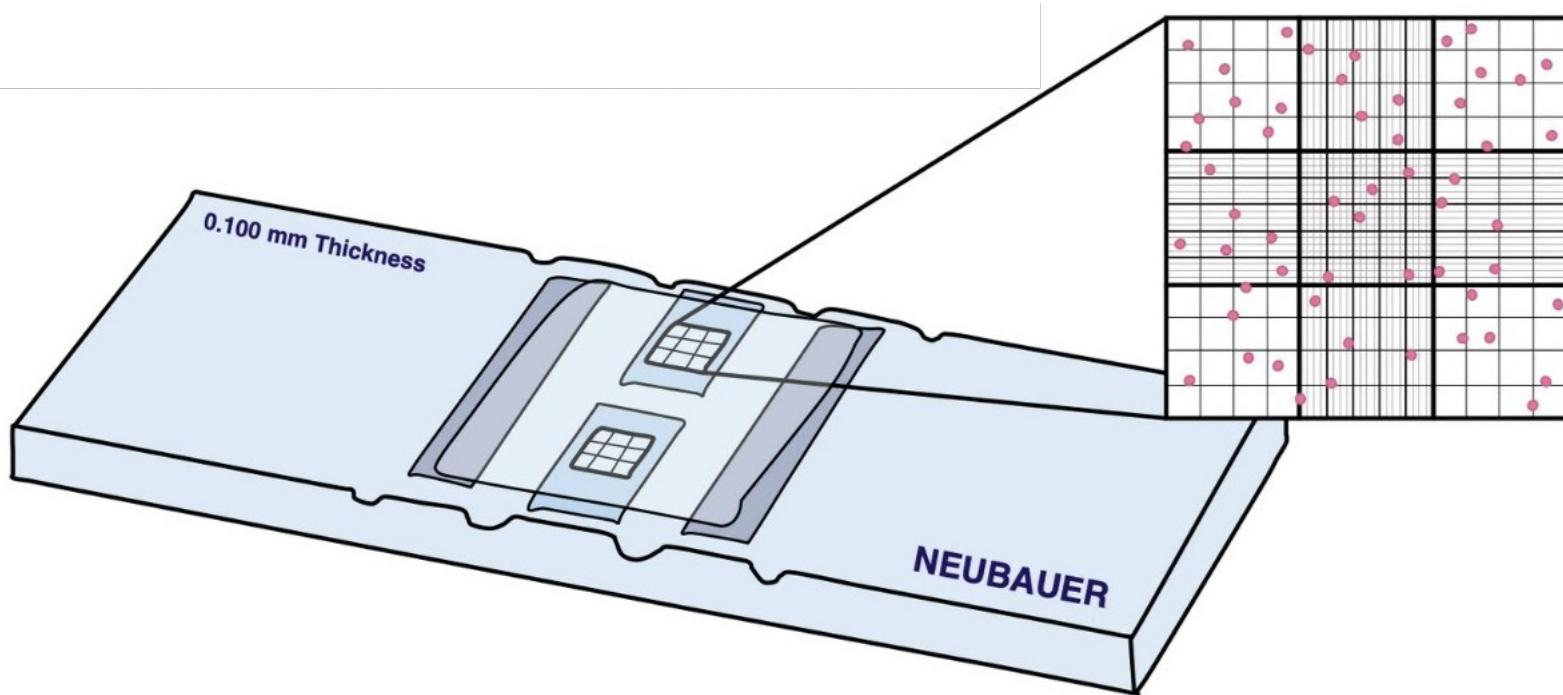
Some basic differences between yeast and bacteria

	Yeasts	Eubacteria
Cell type	Having nucleus (eukaryotic), membrane-bound organelles and cytoskeleton; ribosome 80S	No nucleus (prokaryotic); no membrane-bound organelles and cytoskeleton; ribosome 70S
Plasma membrane	Sterol	No sterol, except <i>Mycoplasma</i>
Cell wall	Glucan; Mannan; Chitin	Peptidoglycan
Spore	a sexual and asexual reproductive structure	Endospore (not for reproduction) but capable of forming vegetative cell
Metabolism	Heterotroph; facultative anaerobic	Heterotroph; autotroph; aerobic, facultative anaerobic, anaerobic

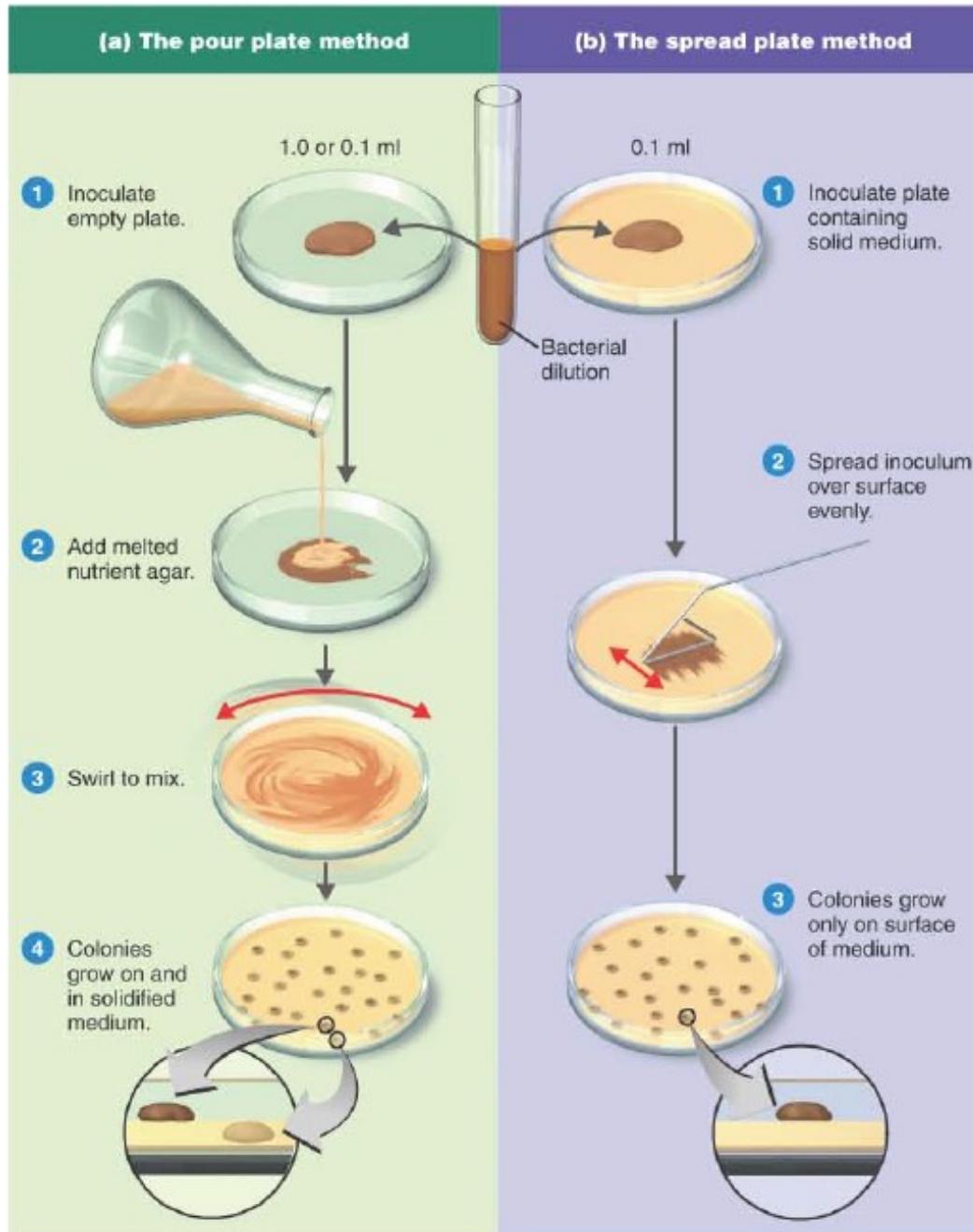
Yeast cell quantification



Yeast cell quantification



Yeast cell quantification



Yeast cell quantification

- Time & materials
- Dead cells