Studies in Biology no. 140

The Biology of Yeast

David R. Berry

The Institute of Biology's
Studies in Biology no. 140

Biology of Yeast

David R. Berry

Senior Lecturer in Applied Microbiology
University of Strathclyde

Edward Arnold

First published 1982
by Edward Arnold (Publishers) Limited
41 Bedford Square, London WC1 3DQ

British Library Cataloguing in Publication Data

Berry, David R.
Biology of yeasts.—(The Institute of Biology's studies in biology, ISSN 0537-9024; no. 140)
1. Yeast fungi
I. Title II. Series
589.2'33 QK617.5

ISBN 0-7131-2838-0

Photoset and printed by Photobooks (Bristol) Ltd

BOOKS IN THE SERIES

1 **Ecological Energetics** Phillipson
2 **Life in the Soil** Jackson & Raw
3 **The Study of Behaviour 2nd edn** Carthy & Howse
4 **An Introduction to Parasitology 2nd edn** Wilson
5 **Plant Taxonomy 2nd edn** Heywood
6 **Microecology** Cloudsley-Thompson
7 **Guts 2nd edn** Morton
8 **The Body Fluids and Their Functions 2nd edn** Chapman
9 **The Electron Microscope in Biology 2nd edn** Grimstone
10 **Translocation in Plants 2nd edn** Richardson
11 **Muscle 2nd edn** Wilkie
12 **Plant Breeding** Lawrence
13 **Chemistry of the Cell** Barker
14 **Plants and Water 2nd edn** Sutcliffe
15 **Developmental Plant Anatomy** Gemmell
16 **Plant Symbiosis** Scott
17 **Fungal Parasitism 2nd edn** Deverall
18 **Population Dynamics 2nd edn** Solomon
19 **Chemical Communication** Ebling & Highnam
20 **Human Genetics and Medicine 2nd edn** Clarke
21 **Cell Division and Heredity** Kemp
22 **Animal Skeletons** Currey
23 **Investigation by Experiment** Heath
24 **Animal Growth and Development** Newth
25 **Animal Photoperiodism** Lofts
26 **Natural History of Infectious Disease** Boycott
27 **The Membranes of Animal Cells 2nd edn** Lockwood
28 **The Biology of Respiration 2nd edn** Bryant
29 **Size and Shape** McNeil Alexander
30 **Cellular Radiobiology** Lawrence
31 **Chloroplasts and Mitochondria** 2nd edn Tribe & Whittaker
32 **Fungal Saprophytism 2nd edn** Hudson
33 **Animal Flight** Pennycuick
34 **How Grasses Grow 2nd edn** Langer
35 **Temperature and Animal Life 2nd edn** Hardy
36 **Nervous Systems** Usherwood
37 **Photosynthesis 3rd edn** Hall & Rao
38 **The Biology of Pollution 2nd edn** Mellanby
39 **How Trees Grow** Morey
40 **Endogenous Plant Growth Substances 2nd edn** Hill
41 **Hair** Ryder
42 **The Structure and Function of Enzymes 2nd edn** Wynn
43 **Introductory Statistics for Biology 2nd edn** Parker
44 **Biology of Aphids** Dixon
45 **Biology of the Food Industry** Barnell
46 **An Introduction to Animal Breeding** Bowman
47 **Experimental Psychology: An Introduction for Biologists** Jeeves
48 **Plants and Mineral Salts 2nd edn** Sutcliffe & Baker
49 **Estuarine Biology** Barnes
50 **Pest Control and its Ecology** van Emden
51 **The Ecology of Small Mammals** Delany
52 **Phytoplankton** Boney
53 **Bone and Biomineralization** Simkiss
54 **The Biology of Plant Phenolics** Walker
55 **Arthropod Vectors of Disease** Busvine
56 **The Biology of Slime Moulds** Ashworth & Dee
57 **Dormancy and the Survival of Plants** Villiers
58 **Ecology of Plants in the Tropics** Janzen
59 **The Optical Microscope in Biology** Bradbury
60 **The Secretion of Milk** Mepham
61 **The Biology of Eucalypts** Pryor
62 **Marine Zooplankton** Wickstead
63 **Homeostasis** Hardy
64 **Diseases in Crops** Wheeler
65 **Plant Tissue Culture** Butcher & Ingram
66 **Lichens as Pollution Monitors** Hawksworth & Rose
67 **Animal Asymmetry** Neville
68 **Phytochrome and Plant Growth** Kendrick & Frankland
69 **Genetics and Adaptation** Ford
70 **Population Cytogenetics** John
71 **A Biology of Locusts** Chapman
72 **The Dynamics of Competition and Predation** Hassell
73 **Mammalian Odours and Pheromones** Stoddart
74 **Decomposition** Mason
75 **Viviparity** Hogarth
76 **Ecology of Fishes in Tropical Waters** Lowe-McConnell
77 **Ecology and Archaeology** Dimbleby
78 **Animals for Man** Bowman
79 **The Biology of Weeds** Hill
80 **Colonization of Industrial Wasteland** Gemmell
81 **The Social Organization of Honeybees** Free
82 **An Introduction to Animal Tissue Culture** Sharp
83 **The Genetic Code** Clark
84 **Lysosomes** Dean
85 **Science and the Fisheries** Cushing
86 **Plants and Temperature** Sutcliffe
87 **Evolution in Modern Biology** Edwards
88 **The Biology of Mucor and its Allies** Ingold
89 **Life in Sandy Shores** Brafield
90 **Antibiotics and Antimicrobial Action** Hammond & Lambert

(see also inside rear cover)

General Preface to the Series

Because it is no longer possible for one textbook to cover the whole field of biology while remaining sufficiently up to date, the Institute of Biology proposed this series so that teachers and students can learn about significant developments. The enthusiastic acceptance of 'Studies in Biology' shows that the books are providing authoritative views of biological topics.

The features of the series include the attention given to methods, the selected list of books for further reading and, wherever possible, suggestions for practical work.

Readers' comments will be welcomed by the Education Officer of the Institute.

1982

Institute of Biology
Queen's Gate
London SW7 5HU

Preface

In this book I have tried to introduce the reader to a wide range of studies which have been and are at present being carried out in one organism, Bakers Yeast. This single fungal species is used by every society in the world for the production of bread or alcoholic beverages, so it is a well recognized organism besides being of considerable economic importance. Yeast is also one of the most favoured organisms for the study of living systems. I have tried to indicate how the scientific study of yeast has arisen out of a need to control traditional fermentation processes such as brewing, and how the present scientific studies are leading to the development of new yeast-based industries. The reader might ask himself how much yeast or yeast products he has consumed to-day.

Although the book is primarily about yeast, the reader will gain some insight into recent techniques and concepts in the field of physiology, cell biology and genetics since the study of one organism like yeast draws on information from all organisms and has significance to all Biological Science.

Glasgow, 1982

D.R.B.

(continued from inside front cover)

91 Boreal Ecology Pruitt
92 Nitrogen Fixation Postgate
93 Haemoglobin Maclean
94 Principles of Human Nutrition Taylor
95 Structure and Function of Viruses Horne
96 Plant Growth Analysis Hunt
97 Man - Hot and Cold Edholm
98 Plant Physiological Ecology Etherington
99 The Physiology of Diving in Man and other Animals Hempleman & Lockwood
100 Cellular Recognition Systems in Plants Heslop-Harrison
101 Sulphur in Biology Anderson
102 The Biology of Helminth Parasites Lyons
103 Sex Hormones and Behaviour Slater
104 Biological Clocks Brady
105 Liver Horner Andrews
106 Aquaculture Reay
107 Pollen and Allergy Knox
108 Animals under Stress Archer
109 Lizards - A Study in Thermoregulation Avery
110 Sexual Incompatibility in Plants Lewis
111 Microorganisms and Man Noble & Naidoo
112 Life at High Altitude Heath & Williams
113 The Nature of Toadstools Ingold
114 Nerves, Brains and Behaviour Messenger
115 Vocal Communication in Birds Catchpole
116 Influences on Animal Growth and Development Batt
117 Collagen: the Anatomy of a Protein Woodhead-Galloway
118 Social Behaviour of Animals Deag
119 Medical Mycology English
120 The Respiration of Higher Plants Op
121 Ecology of Insect-Plant Interactions Edwards & Wratten
122 The Ecology of Streams and Rivers Townsend
123 The Biochemistry of Pollution Ottaway
124 Light and Plant Life Whatley
125 Lipids and Polysaccharides in Biology Furth
126 Bioenergetics of Autotrophs and Heterotrophs Anderson
127 Invertebrate Respiration Wells
128 Immunobiology Inchley
129 Growth Regulators in Crop Production Luckwill
130 Evolution and Pollution Bradshaw & McNeilly
131 The Evolution of Eukaryotic Cells Tribe, Whittaker & Morgan
132 Biological Control of Pests and Weeds Samways
133 Ecological Evaluation for Conservation Spellerberg
134 Molluscs and Man Boyle
135 Neurons and Synapses Jones
136 Biotechnology Smith
137 The Growth of Leaves Dale
138 The Biology of Parasitic Protozoa Baker
139 Ecology of Rocky Shores Brehaut
140 Biology of Yeast Berry

Other volumes in preparation

Nutrition and Health Taylor
Plasmids Day
Animal Taxonomy Goto
Neo-Darwinism Berry

Contents

1 Introduction: the History of Yeast

1.1 Early exploitation of yeast by man

Although man must have been subject to the ravages of disease-causing organisms throughout his evolution, yeast appears to be the first micro-organism to be used by man. Yeast could with justification be viewed as one of the many tools developed by early man. The first records of the use of yeast by man are concerned with the production of a type of acid beer called 'boozah' in 6000 B.C. Egypt. This beer was produced by the fermentation of a dough which was prepared by crushing and kneading germinated barley. The processes for producing beer and wine, and for the production of leaven bread probably developed in parallel over the next few thousand years. In 1200 B.C. Egypt the distinction between leaven and unleaven bread was well established and the use of a portion of yesterday's dough to inoculate today's bread or to inoculate wine fermentations was well established. From Egypt, the technology of brewing and baking passed to Greece and hence to Rome and the Roman Empire.

There is a shortage of records on brewing in the period following the fall of the Roman Empire. It is clear however that in the thirteenth and fourteenth centuries, brewing was well established in the monasteries of northern Europe. It has been reported that between 400 and 500 monasteries in Germany were active in producing beer during this period. As early as 1188, Henry II had levied the first recorded tax on beer in Britain.

The origin of distilled beverages leaves much to conjecture. There are reports of distilled beverages in China in 1000 B.C. and it is clear that whiskey distillation was well established in Ireland in the twelfth century. It is believed that the process of distillation probably came to Europe from the Middle East and this view is supported by the fact that the word alcohol is derived from arabic. Again the production of distilled beverages appears to have been associated with religious establishments and one of the earliest references to whisky in Scotland refers to production by a Friar John Cor in 1494 (Table 1).

1.2 Early scientific study of yeast

A knowledge of the yeast structure was dependent upon the discovery of the microscope and in fact the first description of yeast is attributed to Antonie van Leeuwenhoek in 1680. At this time however, there was no suggestion that the structure described as yeast was a living organism. It is difficult to establish who were the first scientists to suggest that yeasts were living organisms which caused the alcoholic fermentation observed in wines and beer. Vitalistic theories of

Table 1 Some key steps in the technological utilization and scientific study of brewers yeast. (Abstracted from *Yeast Technology* by G. Reed and H. J. Peppler, A.V.I. Publishing Company, 1973, and other sources.)

6000 B.C.	Evidence of brewing in Egypt
1000 B.C.	Consumption of potable distilled spirits in China
1192 A.D.	Whiskey production in Ireland
1200–1300	Breweries established throughout northern Europe
1680	Observation of yeast by Antonie van Leeuwenhoek
1832	Yeast recognized as fungi by Persoon and Fries
1838	Brewing yeast named *Saccharomyces cerevisiae* by Meyer
1839	Yeast spores described by Schwann
1863	Role of yeast in fermentations established by Pasteur
1866	Life cycle of yeast demonstrated by de Barry
1881	Pure cultures obtained by Hansen
1896	Scientific system for classifying yeast published by Hansen
1897	Fermentation by cell free extracts of yeast reported by Buchner
1934	Alternation of haploid and diploid phases in life cycle of yeast demonstrated by Winge
1943	Heterothallism in *Saccharomyes* reported by Lindegren

fermentation were proposed in the late eighteenth century and in 1818 Erxleben suggested that yeast was responsible for alcoholic fermentations. However, it is generally agreed that it was the work of Pasteur published in his *Etudes sur Vin*, in 1866 which established beyond doubt the role of yeasts in the fermentation of sugars to alcohol. This work represents a milestone in the development of microbiology. Another important milestone was the establishment of pure yeast cultures from single cell isolates by Hansen in 1881. The use of pure cultures has been fundamental to the development of the taxonomy and physiology of yeast and other micro-organisms. In 1897, Buchner obtained a cell free extract by grinding yeast which was capable of fermenting sugars to alcohol, and by doing so established one of the foundation stones of modern biochemistry. Subsequent work in this area made a significant contribution to the elucidation of the Embden-Meyerhof-Parnas (EMP) pathway. Since this time, yeast has been a favoured organism for a wide range of physiological and biochemical studies. Of special interest to those interested in alcoholic beverages was the establishment by Ehrlich in 1906 of the relationship between amino acid metabolism and the production of fusel alcohols, a key group of organoleptic compounds produced by yeast.

Early developments in the field of microbial genetics also arose out of studies on yeast. The alternation of haploid and diploid phases in the life cycle of yeast was established by Winge (1935) who subsequently went on to demonstrate the Mendelian segregation of genes during sexual reproduction in yeast. These studies opened the way for extensive studies in yeast genetics which have made a major contribution to our understanding of the nature of the genetic material and the mechanism of inheritance in eukaryotic micro-organisms.

1.3 Development of yeast classification

Although the characteristic budding form of yeast has been recognized since the description by van Leeuwenhoek in 1680, a more precise description and identification of yeasts has always presented a problem. Since the vegetative forms of most yeasts do not have any distinctive morphological characteristics, they are not readily identifiable by direct observation. Initially, the name *Saccharomyces* was applied to all yeasts isolated from alcoholic beverages and three species were recognized by Meyen (1837) by their origin; *Saccharomyces vini* from wine, *S. cerevisiae* from beer and *S. pomorum* from cider. Yeast sexual spores were recognized by Schwann in 1837 but only in 1870 was the genus *Saccharomyces* restricted to those yeasts which produced spores.

1.4 Brewers and bakers yeast

The genus *Saccharomyces* contains some forty species, each of which produce spherical to ellipsoidal cells by budding, produce ascospores in asci and are capable of the efficient conversion of sugars to alcohol. The most important *Saccharomyces* species in brewing was isolated in pure culture, and described by Hansen as *Saccharomyces cerevisiae* var *ellipsoideus* (Hansen) Dekker. Strains classified as *S. cerevisiae* have been widely used for brewing, distilling, wine making, the production of bakers yeast and biomass production. However the species has been defined in different ways at different times. A recent monograph published by Barnett, Payne and Yarrow (1900) includes in *S. cerevisiae* strains which have been classified in eighteen different species in previous systems of classification. One of these, *S. carlsbergensis*, is of particular interest, since it is the 'bottom yeast' used for the fermentation of lager. It is recognized by its ability to metabolize the sugar melibiose. Although widely referred to a *S. carlsbergensis* in the brewing industry, it was reclassified as *S. uvarum* by Van de Walt in 1970 and has been more recently included in the species *S. cerevisiae* by Barnett, Payne and Yarrow.

2 The Architecture of the Yeast Cell

2.1 Cell morphology

The cells of *Saccharomyces cerevisiae* are round, ovoid or ellipsoidal in shape and vary from 2.5–10 μm in width and 4.5–21 μm in length. Unstained cells exhibit little detail under the light microscope and even when inclusions in the cytoplasm are recognizable, it is difficult to know whether they represent vacuoles, granules or nuclei. Although more information can be obtained by using specific stains, it is only since the advent of the electron microscope that a clear picture of the yeast cell has emerged. The characteristic features of a typical yeast cell are shown in Fig. 2–1. It can be seen that the cell is bounded by a thick cell wall. Inside this it is possible to recognize many of the features of a typical cell; a plasmalemma, a nucleus, mitochondria, endoplasmic reticulum, vacuoles, vesicles and granules. The structure and function of these different structures will be considered in the remainder of this chapter.

The distinguishing feature of a growing population of yeast cells is the presence of the buds which are produced when the cell divides. The daughter cell is initiated as a small bud which increases in size throughout most of the cell cycle, until it is the same size as the mother cell. Most growth in yeast occurs during bud formation, so the bud is more or less the same size as the mature cell

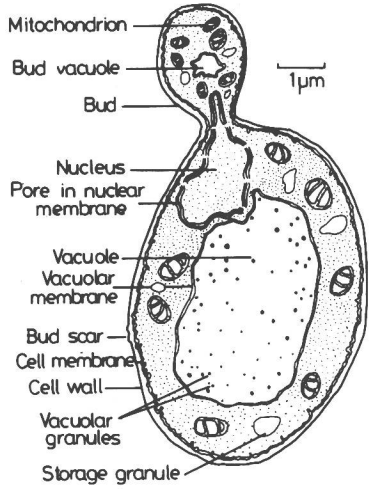

Fig. 2–1 Section through typical yeast cell showing the main features of the cell and their distribution. (Reproduced from Webster, 1980, *Introduction to Fungi*, p. 273. Cambridge University Press.)

before it separates. Cell separation may occur soon after cell division; however often new rounds of cell division take place before cell separation has occurred, so groups of cells are produced. The site of cell separation is marked on the mother cell by a structure referred to as the bud scar and on the daughter cell by the birth scar. These scars cannot be seen under the light microscope but can be seen using fluorescence microscopy after staining with fluorescent stains such as calcafluor or primulin. Bud scars and birth scars also show up as very distinct structures in scanning electron micrographs. No two buds arise at the same site on the cell wall in *Saccharomyces cerevisiae*, so each time a bud is produced a new bud scar is produced in the cell wall of the mother cell. By counting the number of bud scars, it is possible to establish the number of buds which have been produced by a particular cell. This can be used as a measure of the age of the cell. In any yeast population, 50% of the cells were produced by the last generation of cell divisions so possess a birth scar but no bud scar. Of the remaining 50%, 25% have one bud scar, 12.5% two bud scars and 12.5% more than two bud scars (Fig. 2–3c)

In some strains, cells growing in liquid culture adhere together to form clumps which settle to the bottom of the growth vessel. This phenomenon, which is referred to as flocculence, is of considerable importance in the brewing industry.

2.2 The cell wall

The cell wall is a rigid structure which is 25 nm thick and constitutes approximately 25% of the dry weight of the cell. Chemical analysis of the cell wall, indicates that the major components are glucan and mannan; however chitin and protein are also present. Glucan is a complex branched polymer of glucose units and is located in the inner layer of the yeast cell wall adjacent to the plasmalemma (see section 2.3). It appears to be the major structural component of the cell wall, since removal of the glucan results in a total disruption of the cell wall. Mannan, which is a complex polymer of mannose occurs mainly in the outer layers of the cell wall. Since it is possible to remove the mannan without altering the general shape of the cell, it appears that it is not essential to the integrity of the cell wall. The third carbohydrate component referred to; chitin, is a polymer of N,acetyl-glucosamine and is found in the cell wall of *S. cerevisiae* associated with the bud scars. Isolation of the bud scars by treating the cell wall with appropriate lytic enzymes, has shown that the chitin is arranged in a ring around the bud scar. Protein constitutes 10% of the dry weight of the cell wall. At least some of this protein is in the form of wall bound enzymes. Several enzymes have been described as being associated with the cell wall in yeast, including glucanase and mannanase, which are probably involved in the softening of the cell wall to permit bud formation; invertase, alkaline phosphatase and lipase. Several of these enzymes, e.g. invertase are manno-proteins and contain up to 50% of mannan, as an integral part of the enzyme molecule. Much of the remaining protein in the cell wall is also associated with mannan, so it is possible that this plays a structural rather than enzymic role in the cell wall. The detailed organization of the cell wall is not fully understood,

but current theories favour a three-layered structure in which the inner glucan layer is separated from the outer mannan layer by a layer which is rich in protein (Fig. 2–2a).

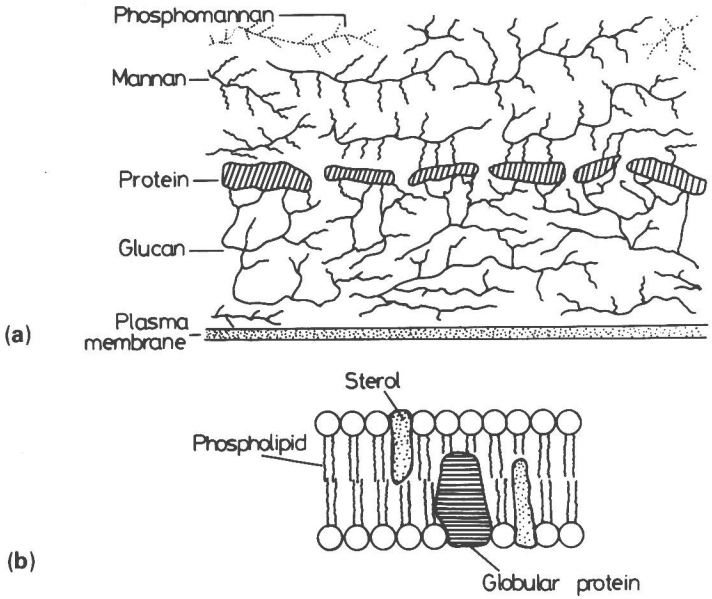

Fig. 2–2 (a) Diagrammatic representation of the structure of the yeast cell wall indicating the distribution of glucan and mannan in different layers. (b) Diagrammatic representation of a phospholipid membrane such as the plasmalemma. (Reproduced from Rose, A.H., 1976, *Chemical Microbiology*, p. 30. Butterworth, London.)

2.3 The cell membrane

The cell membrane or plasmalemma of the yeast cell can be observed using electron microscopy as a three-layered structure which is closely associated with the inner surface of the cell wall. It usually has a smooth appearance but at certain stages of the growth of the cell, invaginations can be seen. An understanding of the chemical composition of the plasmalemma requires isolation techniques which produce plasmalemma membrane free of other cellular components, including other membranes. One technique involves the formation of protoplasts; cells from which the cell wall has been removed by treatment with suitable lytic enzymes such as the snail juice enzyme, which is a preparation of lytic enzymes obtained from the gut of the snail *Helix pomatia*. Protoplasts remain intact if they are held in an isotonic solution of sugars but are readily burst when placed in more dilute suspension media. Different organelles, including the plasmalemma, can be obtained by centrifugation of a preparation of burst protoplasts. An alternative technique is to mechanically disrupt the cells first and remove the cell contents from the wall preparation by washing. The

plasmalemma remains attached to the cell wall and can be liberated by dissolving the cell wall with lytic enzymes.

The plasmalemma is composed of lipids and proteins in more or less equal amounts, together with a small amount of carbohydrate. The main lipids present are mono-, di- and triglycerides, glycerophosphatides and sterols such as ergosterol and zymosterol. The nature of the protein in the plasmalemma is less well understood but probably includes the enzymes which are involved in the uptake of sugars and amino acids. Models of the structure of the plasmalemma have been presented (see Fig. 2–2b). Phospholipids are amphipathic molecules; that is each molecule consists of two regions, one of which is hydrophobic, i.e. repelled by water and another which is hydrophilic, i.e. attracted to water. They are believed to be arranged in such a manner that the hydrophilic parts of the molecule lie on the outside of the membrane and the hydrophobic lie on the inside of the membrane. The protein molecules may be arranged on the surface of the membrane or pass through the membrane.

The plasmalemma is a major organelle in the cell. It acts as a permeability barrier around the contents of the cell and controls the transport of solutes into and out of the cell. Strong evidence has also been presented that the plasmalemma is involved in the control of cell wall biosynthesis in yeast. *Saccharomyces cerevisiae* is unusual in that it cannot synthesize certain unsaturated fatty acids and sterols when it is grown in strictly anaerobic conditions, so these must be supplied in the medium if growth is to continue. Since the fatty acids and sterols supplied are incorporated into the cell membranes, it is possible to influence the chemical composition of the plasmalemma by feeding different fatty acids and sterols. Using this technique, it has been shown that changes in the lipid composition of the membrane affect the osmotic properties, the temperature sensitivity and the solute uptake characteristics of the cell.

2.4 The nucleus

The nucleus can be recognized by phase contrast microscopy and is usually situated between the vacuole and the bud. Chromatic bodies can be recognized in the nucleus using specific stains such as acid fuchsin or giemsa. However, knowledge of the studies of the yeast nucleus is limited, since individual chromosomes are very small, similar in size to a chromosome of *Escherichia coli* and not recognizable as discrete structures either by light microscopy or electron microscopy. The nuclear membrane remains intact throughout the cell cycle. It is visible in electron micrographs as a double membrane which is perforated at intervals with pores. Associated with the nuclear membrane is a structure referred to as a plaque which appears to function in a similar manner to the centrioles of animal cells. The characteristic structure of a plaque is a multilayered disc from which microtubules extend into both the nucleus and the cytoplasm. These plaques are considered to represent the spindle apparatus of the yeast nucleus and their behaviour has been monitored to follow the different stages of nuclear division (Chapter 4) (Fig. 2–3d).

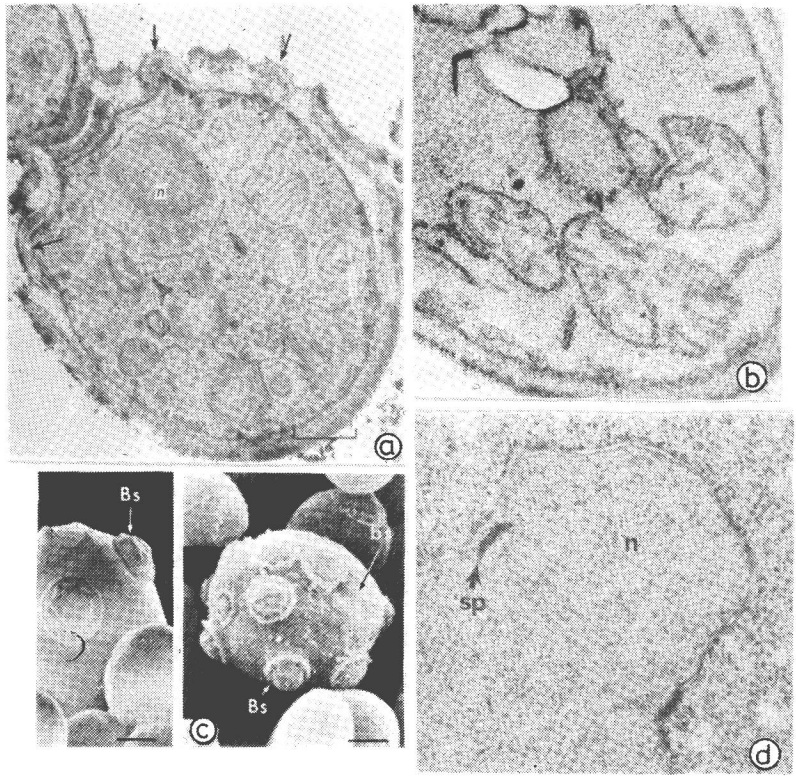

Fig. 2–3 Electron micrographs of yeast, showing sub-cellular structures. (**a**) Section of budding cell showing bud scars (arrows), nucleus (n), and developing cell septum. (Reproduced from Marchant and Smith, 1968, *J. Gen Micro.*, **53**, 168.) (**b**) Section of aerobic cell showing mitochondria. (Reproduced from Smith *et al.*, 1969, *J. Gen Micro.*, **56**, 54.) (**c**) Scanning electron micrograph showing bud scars (Bs) and birth scars (bs). (Reproduced from Belin, 1972, *Antonie van Leeuwenhoek*, **38**, 343.) (**d**) Section showing spindle plaques (sp) connected by microtubules during nuclear division. (Reproduced from Byers and Goetsch, 1973, *Cold Spring Harbour Symp.*, **38**, 123.)

2.5 Mitochondria

Mitochondria are readily recognizable in electron micrographs of aerobically grown yeast (see Chapter 3) as spherical or rod-shaped structures surrounded by a double membrane. They contain cristae which are formed by the folding of the inner membrane (Fig. 2–3b).

A considerable amount of work has been carried out on the structure of the mitochondrion and the distribution of the many mitochondrial enzymes in the membranes and the matrix of the mitochondrion. Most of the enzymes of the tricarboxylic acid cycle are present in the matrix of the mitochondrion, whereas the enzymes involved in electron transport and oxidative phosphorylation are

associated with the inner membrane, including the cristae.

At one time it was considered that mitochondria were absent from anaerobically grown or catabolite repressed yeast (see Chapter 3) since they could not be detected and also because such cells lacked many of the enzymes associated with mitochondria. More recently, the use of freeze-etching techniques has indicated that the apparent absence of mitochondria was due to inadequate fixation techniques. Cells grown anaerobically in the absence of lipids have very simple mitochondria, consisting of an outer double membrane but lacking cristae. The addition of lipids such as oleic acid and ergosterol results in the development of cristae. The development of the mitochondrion is influenced by the lack of oxygen, the presence of lipids and the level of glucose in the medium. Thus, contrary to previous ideas, there is a change in the structure of mitochondria upon transfer from anaerobic to aerobic conditions but no *de novo* generation of mitochondria.

2.6 Other cytoplasmic structures

The cytoplasm of the yeast cell contains a system of double membranes known as the endoplasmic reticulum. Some of these membranes are associated with ribosomes as in other organisms; however, the endoplasmic reticulum appears to be involved in many other cellular activities. The relationship between endoplasmic reticulum and other organelles is not clear; however, there is continuity between the endoplasmic reticulum, the outer membrane of the mitochondrion and the plasmalemma. The endoplasmic reticulum is also involved in the formation of vesicles which are present in the cell. In this it behaves in a manner akin to the Golgi apparatus of some other organisms. It is not clear however, whether a true Golgi apparatus is present in yeast; membranous discs have been observed in yeast cells but they are few in number and not clearly recognizable as a Golgi apparatus.

Lipid granules are also present in the cytoplasm and again these appear to be derived from the endoplasmic reticulum.

Mature yeast cells contain a large vacuole; however at the point in the cell cycle when the bud formation is initiated, the vacuole appears to fragment into smaller vacuoles which become distributed between the mother cell and the bud. Later on in the cell cycle, these small vacuoles fuse again to produce a single vacuole in the mother and daughter cell.

The function of the vacuole is not well established. Evidence has been presented that it contains hydrolytic enzymes, polyphosphates, lipids and low molecular weight cellular intermediates, and metal ions. It may act as a storage reservoir for nutrients and for hydrolytic enzymes.

The technical problems of isolating and characterizing the different membrane components of yeast are considerable. Vesicles, vacuoles and other organelles are very fragile and easily disrupted. Since fragments of membrane from different organelles are almost impossible to separate, it is perhaps not surprizing that our understanding of the functional relationships between several of these structures is limited.

3 Nutrition and Metabolism of Yeast

3.1 Nutritional requirements

Although brewers and bakers yeast is probably the most readily recognized micro-organism for the layman, microbiologists have had difficulties in defining the species *Saccharomyces cerevisiae* in precise terms (Chapter 1). Since current opinion tends towards including several types of yeast which were previously classified as separate species, it is not easy to generalize on the nutritional requirements of *S. cerevisiae*. All the strains classified as *S. cerevisiae* can grow aerobically on glucose, sucrose, maltose and trehalose and fail to grow on lactose and cellobiose. However, growth on certain other sugars is variable, e.g. strains previously referred to as *S. carlsbergensis* or *S. uvarum* can metabolize raffinose completely and strains previously known as *S. diastaticus* and *S. chevalieri*, can grow on starch. The ability of yeasts to use different sugars can differ depending upon whether the cells are growing aerobically or anaerobically. Some *S. cerevisiae* strains cannot grow anaerobically on sucrose or trehalose.

All *S. cerevisiae* strains can utilize ammonia and urea as the sole nitrogen source but cannot utilize nitrate since they lack the ability to reduce it to ammonium ions. They can also utilize most amino acids, small peptides and bases as a nitrogen source. Histidine, glycine, cystine and lysine are however, not readily utilized. *S. cerevisiae* does not excrete proteases so extracellular protein cannot be metabolized.

The requirements of *S. cerevisiae* for growth factors such as pantothenate, biotin, thiamin, pyridoxine, niacin, folic acid and para-aminobenzoic acid are not clear, probably because different strains have different requirements, and also because the requirement for a growth factor can be influenced by other growth conditions. Yeasts also have a requirement for phosphorus, which is assimilated as the dihydrogen phosphate ion ($H_2PO_4^-$), and sulphur which can be assimilated either as sulphate (SO_4^{2-}) or as organic sulphur compounds such as methionine and cystine. The potassium requirement of yeast can be partly replaced by other alkali metals and even ammonium. Other metals required for good yeast growth include magnesium, calcium, zinc, iron and copper.

3.2 Growth of yeast

3.2.1 Batch culture

In many industrial processes such as brewing, distilling and wine making, yeast is grown in a medium which is rich in sugars (see Chapter 8). In these circumstances, growth is virtually anaerobic and the sugars assimilated are metabolized to carbon dioxide and ethanol by the process known as ethanol