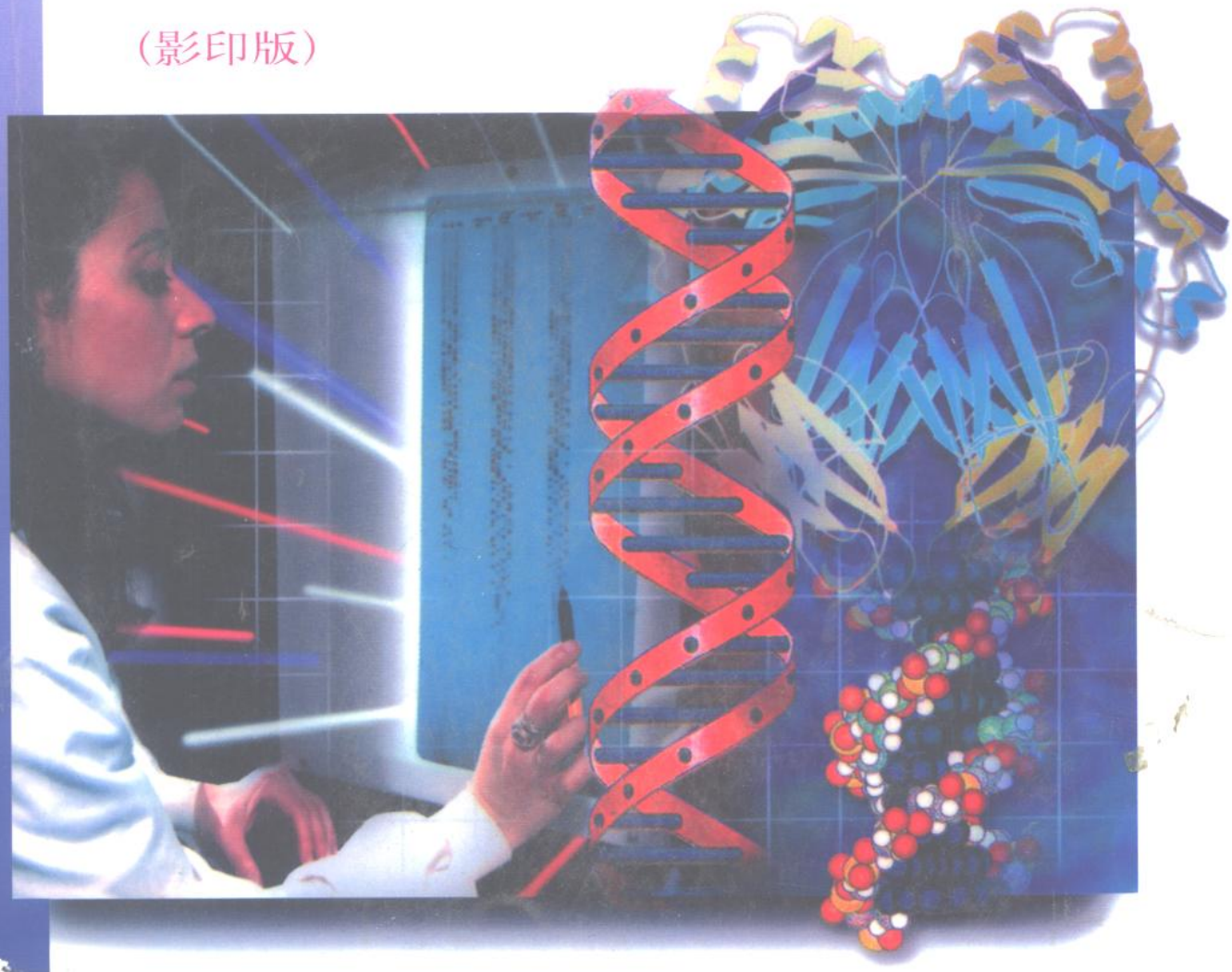
现代生物学精要速览

Instant Notes in MOLECULAR BIOLOGY

分子生物学

（影印版）



P.C.Turner, A.G.McLennan,
A.D.Bates & M.R.H.White

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P.C. Turner, A.G. McLennan,
A.D. Bates & M.R.H. White

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University of Liverpool, Liverpool, UK

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内 容 简 介

本套丛书是国外优秀教材畅销榜的上榜教材，面向大学生，由英国著名大学具丰富教学经验的一流教授编写。它以一种风格独特的描述方式，全面、系统地概括了学科的核心内容和前沿动态，并以一种便于学习、利于复习的形式，使学生能快速、准确地掌握知识，很好地指导学习和考试。书中英文使用最为自然、易懂的语句，是提高专业外语的最佳套书。本书是该系列中的分子生物学分册，共约 20 个章节。

P. C. Turner, A. G. McLennan, A. D. Bates and M. R. H. White

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ABBREVIATIONS

ADP	adenosine 5′-diphosphate
AIDS	acquired immune deficiency syndrome
AMP	adenosine 5′-monophosphate
ARS	autonomously replicating sequence
ATP	adenosine 5′-triphosphate
BER	base excision repair
bp	base pairs
BRF	TFIIB-related factor
BUdR	bromodeoxyuridine
bZIP	basic leucine zipper
CDK	cyclin-dependent kinase
cDNA	complementary DNA
CJD	Creutzfeld–Jakob disease
CRP	cAMP receptor protein
CSF-1	colony-stimulating factor-1
CTD	carboxyl-terminal domain
Da	Dalton
dNTP	deoxynucleoside triphosphate
ddNTP	dideoxynucleoside triphosphate
DMS	dimethyl sulfate
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dsDNA	double-stranded DNA
EDTA	ethylenediamine tetraacetic acid
EF	elongation factor
ENU	ethylnitrosourea
ER	endoplasmic reticulum
ESI	electrospray ionization
ETS	external transcribed spacer
FADH	reduced flavin adenine dinucleotide
β-gal	β-galactosidase
GMO	genetically modified organism
GTP	guanosine 5′-triphosphate
HIV	human immunodeficiency virus
HLH	helix–loop–helix
hnRNA	heterogeneous nuclear RNA
hnRNP	heterogeneous nuclear ribonucleoprotein
HSP	heat-shock protein
HSV-1	herpes simplex virus-1
IF	initiation factor
IHF	integration host factor
IPTG	isopropyl-β-D-thiogalactopyranoside
IS	insertion sequence
ITS	internal transcribed spacer
JAK	Janus activated kinase
kb	kilobase pairs in duplex nucleic acid, kilobases in single-stranded nucleic acid
kDa	kiloDalton
LAT	latency-associated transcript
LTR	long terminal repeat
MALDI	matrix-assisted laser desorption/ionization
MCS	multiple cloning site
MMS	methylmethane sulfonate
MMTV	mouse mammary tumor virus
mRNA	messenger RNA
NAD^+	nicotinamide adenine dinucleotide
NER	nucleotide excision repair
NLS	nuclear localization signal
NMN	nicotinamide mononucleotide
NMR	nuclear magnetic resonance
nt	nucleotide
NTP	nucleoside triphosphate
ORC	origin recognition complex
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PAP	poly(A) polymerase
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PTH	phenylthiohydantoin
RBS	ribosome-binding site
RER	rough endoplasmic reticulum
RF	replicative form
RNA	ribonucleic acid
RNA Pol I	RNA polymerase I
RNA Pol II	RNA polymerase II
RNA Pol III	RNA polymerase III
RNase A	ribonuclease A
RNase H	ribonudease H
RNP	ribonucleoprotein
ROS	reactive oxygen species
RP-A	replication protein A
rRNA	ribosomal RNA
RT	reverse transcriptase

RT–PCR	reverse transcriptase–polymerase chain reaction
SAM	*S*-adenosylmethionine
SDS	sodium dodecyl sulfate
SL1	selectivity factor 1
snRNA	small nuclear RNA
snRNP	small nuclear ribonucleoprotein
SRP	signal recognition particle
Ssb	single-stranded binding protein
ssDNA	single-stranded DNA
SV40	simian virus 40
TAF	TBP-associated factor
TBP	TATA-binding protein
α-TIF	α-*trans*-inducing factor
Tris	tris(hydroxymethyl)amino-methane
tRNA	transfer RNA
UBF	upstream binding factor
UCE	upstream control element
URE	upstream regulatory element
UV	ultraviolet
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
XP	xeroderma pigmentosum
YAC	yeast artificial chromosome
YEp	yeast episomal plasmid

PREFACE

The last 20 years have witnessed a revolution in our understanding of the processes responsible for the maintenance, transmission and expression of genetic information at the molecular level – the very basis of life itself. Of the many technical advances on which this explosion of knowledge has been based, the ability to remove a specific fragment of DNA from an organism, manipulate it in the test tube, and return it to the same or a different organism must take pride of place. It is around this essence of recombinant DNA technology, or genetic engineering to give it its more popular title, that the subject of molecular biology has grown. Molecular biology seeks to explain the relationships between the structure and function of biological molecules and how these relationships contribute to the operation and control of biochemical processes. Of principal interest are the macromolecules and macromolecular complexes of DNA, RNA and protein and the processes of replication, transcription and translation. The new experimental technologies involved in manipulating these molecules are central to modern molecular biology. Not only does it yield fundamental information about the molecules, but it has tremendous practical applications in the development of new and safe products such as therapeutics, vaccines and foodstuffs, and in the diagnosis of genetic disease and in gene therapy.

An inevitable consequence of the proliferation of this knowledge is the concomitant proliferation of comprehensive, glossy textbooks, which, while beautifully produced, can prove somewhat overwhelming in both breadth and depth to first and second year undergraduate students. With this in mind, *Instant Notes in Molecular Biology* aims to deliver the core of the subject in a concise, easily assimilated form designed to aid revision. The book is divided into 19 sections containing 70 topics. Each topic consists of a 'Key Notes' panel, with extremely concise statements of the key points covered. These are then amplified in the main part of the topic, which includes simple and clear black and white figures, which may be easily understood and reproduced. To get the best from this book, material should first be learnt from the main part of the topic; the Key Notes can then be used as a rapid revision aid. Whilst there is a reasonably logical order to the topics, the book is designed to be 'dipped into' at any point. For this reason, numerous cross-references are provided to guide the reader to related topics.

The contents of the book have been chosen to reflect both the major techniques used and the conclusions reached through their application to the molecular analysis of biological processes. They are based largely on the molecular biology courses taught by the authors to first and second year undergraduates on a range of biological science degree courses at the University of Liverpool. Section A introduces the classification of cells and macromolecules and outlines some of the methods used to analyze them. Section B considers the basic elements of protein structure and the relationship of structure to function. The structure and physico-chemical properties of DNA and RNA molecules are discussed in Section C, including the complex concepts involved in the supercoiling of DNA. The organization of DNA into the intricate genomes of both prokaryotes and eukaryotes is covered in Section D. The related subjects of mutagenesis, DNA replication, DNA recombination and the repair of DNA damage are considered in Sections E and F.

Section G introduces the technology available for the manipulation of DNA sequences. As described above, this underpins much of our detailed understanding of the molecular mechanisms of cellular processes. A simple DNA cloning scheme is used to introduce the basic methods. Section H describes a number of the more sophisticated cloning vectors which are used for a variety of purposes. Section I considers the use of DNA libraries in the isolation of new gene sequences, while Section J covers more complex and detailed methods, including DNA sequencing and the analysis of cloned sequences. This section concludes with a discussion of some of the rapidly expanding applications of gene cloning techniques.

The basic principles of gene transcription in prokaryotes are described in Section K, while Section L gives examples of some of the sophisticated mechanisms employed by bacteria to control specific

gene expression. Sections M and N provide the equivalent, but necessarily more complex, story of transcription in eukaryotic cells. The processing of newly transcribed RNA into mature molecules is detailed in Section O, and the roles of these various RNA molecules in the translation of the genetic code into protein sequences are described in Sections P and Q. The contributions that prokaryotic and eukaryotic viruses have made to our understanding of molecular information processing are detailed in Section R. Finally, Section S shows how the study of viruses, combined with the knowledge accumulated from many other areas of molecular biology is now leading us to a detailed understanding of the processes involved in the development of a major human affliction – cancer.

This book is not intended to be a replacement for the comprehensive mainstream textbooks; rather, it should serve as a direct complement to your lecture notes to provide a sound grounding in the subject. The major texts, some of which are listed in the Further Reading section at the end of the book, can then be consulted for more detail on topics specific to the particular course being studied. For those of you whose fascination and enthusiasm for the subject has been sufficiently stimulated, the reading list also directs you to some more detailed and advanced articles to take you beyond the scope of this book. Inevitably, there have had to be omissions from *Instant Notes in Molecular Biology* and we are sure each reader will spot a different one. However, many of these will be covered in other titles in the Instant Notes series, such as the companion volume, *Instant Notes in Biochemistry*.

Phil Turner, Sandy McLennan, Andy Bates and Mike White

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A1 CELLULAR CLASSIFICATION

Key Notes

Eubacteria

Structurally defined as prokaryotes, these cells have a plasma membrane, usually enclosed in a rigid cell wall, but no intracellular compartments. They have a single, major circular chromosome. They may be unicellular or multicellular. *Escherichia coli* is the best studied eubacterium.

Archaea

The Archaea are structurally defined as prokaryotes but probably branched off from the eukaryotes after their common ancestor diverged from the eubacteria. They tend to inhabit extreme environments. They are biochemically closer to eubacteria in some ways but to eukaryotes in others. They also have some biochemical peculiarities.

Eukaryotes

Cells of plants, animals, fungi and protists possess well-defined subcellular compartments bounded by lipid membranes (e.g. nuclei, mitochondria, endoplasmic reticulum). These organelles are the sites of distinct biochemical processes and define the eukaryotes.

Differentiation

In most multicellular eukaryotes, groups of cells differentiate during development of the organism to provide specialized functions (e.g. as in liver, brain, kidney). In most cases, they contain the same DNA but transcribe different genes. Like all other cellular processes, differentiation is controlled by genes. Co-ordination of the activities of different cell types requires communication between them.

Related topics

Subcellular organelles (A2)
Prokaryotic and eukaryotic chromosome structure (Section D)
Bacteriophages and eukaryotic viruses (Section R)

Eubacteria

The **Eubacteria** are one of two subdivisions of the **prokaryotes**. Prokaryotes are the simplest living cells, typically 1–10 μm in diameter, and are found in all environmental niches from the guts of animals to acidic hot springs. Classically, they are defined by their structural organization (*Fig. 1*). They are bounded by a **cell (plasma) membrane** comprising a lipid bilayer in which are embedded proteins that allow the exit and entry of small molecules. Most prokaryotes also have a rigid cell wall outside the plasma membrane which prevents cell **lysis** (bursting) in environments of low osmolarity. The cell interior (**cytoplasm** or **cytosol**) contains a single, circular chromosome compacted into a **nucleoid** and attached to the membrane (see Topic D1), and often plasmids [small deoxyribonucleic acid (DNA) molecules with limited genetic information, see Topic G2], ribonucleic acid (RNA), ribosomes (the sites of protein synthesis, see Section Q) and most of the proteins which perform the metabolic reactions of the cell. Some of these proteins are attached to the plasma membrane, but there are no distinct **subcellular organelles** as in **eukaryotes** to compartmentalize different

parts of the metabolism. The surface of a prokaryote may carry **pili**, which allow it to attach to other cells and surfaces, and **flagella**, whose rotating motion allows the cell to swim. Most prokaryotes are unicellular; some, however, have multicellular forms in which certain cells carry out specialized functions. The **Eubacteria** differ from the **Archaea** mainly in their biochemistry. The eubacterium *Escherichia coli* has a **genome size** (DNA content) of 4600 kilobase pairs (kb) which is sufficient genetic information for about 3000 proteins. Its molecular biology has been studied extensively. The genome of the simplest bacterium, *Mycoplasma genitalium*, has only 580 kb of DNA and encodes just 470 proteins. It has a very limited metabolic capacity.

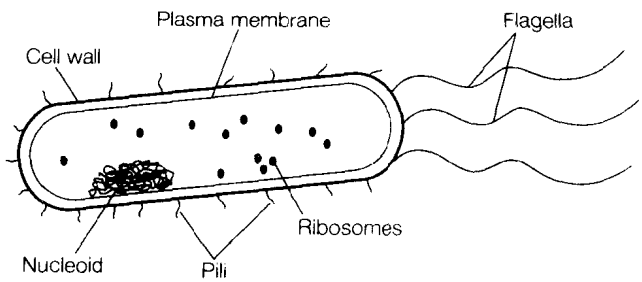

Fig. 1. Schematic diagram of a typical prokaryotic cell.

Archaea

The **Archaea**, or **archaebacteria**, form the second subdivision of the prokaryotes and tend to inhabit extreme environments. Structurally, they are similar to eubacteria. However, on the basis of the evolution of their ribosomal RNA (rRNA) molecules (see Topic O1), they appear as different from the eubacteria as both groups of prokaryotes are from the eukaryotes and display some unusual biochemical features, for example ether in place of ester linkages in membrane lipids (see Topic A3). The 1740 kb genome of the archaeon *Methanococcus jannaschii* encodes a maximum of 1738 proteins. Comparisons reveal that those involved in energy production and metabolism are most like those of eubacteria while those involved in replication, transcription and translation are more similar to those of eukaryotes. It appears that the Archaea and the eukaryotes share a common evolutionary ancestor which diverged from the ancestor of the Eubacteria.

Eukaryotes

Eukaryotes are classified taxonomically into four kingdoms comprising **animals**, **plants**, **fungi** and **protists** (algae and protozoa). Structurally, eukaryotes are defined by their possession of membrane-enclosed organelles (*Fig. 2*) with specialized metabolic functions (see Topic A2). Eukaryotic cells tend to be larger than prokaryotes: 10–100 μm in diameter. They are surrounded by a plasma membrane, which can have a highly convoluted shape to increase its surface area. Plants and many fungi and protists also have a rigid cell wall. The cytoplasm is a highly organized gel that contains, in addition to the organelles and ribosomes, an array of protein fibers called the **cytoskeleton** which controls the shape and movement of the cell and which organizes many of its metabolic functions. These fibers include **microtubules**, made of **tubulin**, and **microfilaments**, made of **actin** (see Topic A4). Many eukaryotes are multicellular, with groups of cells undergoing **differentiation** during development to form the specialized tissues of the whole organism.

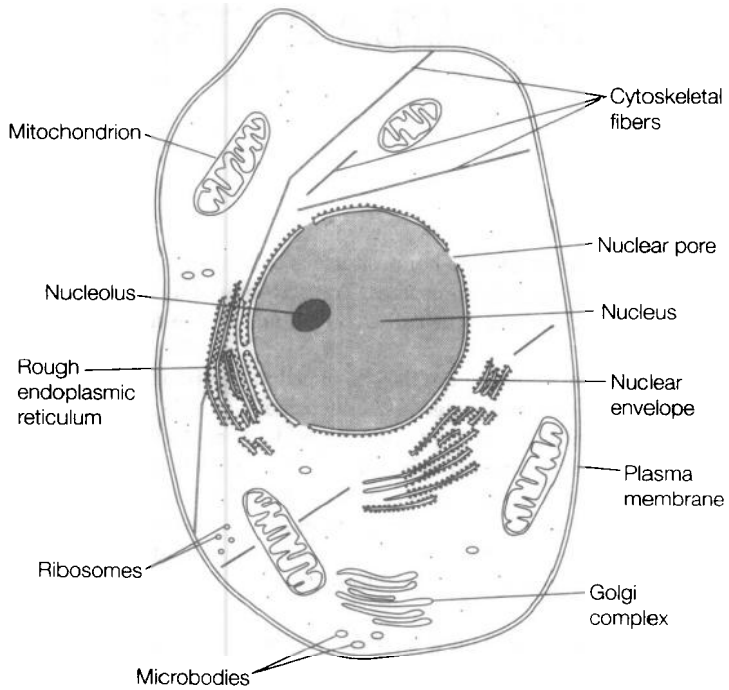

Fig. 2. Schematic diagram of a typical eukaryotic cell.

Differentiation

When a cell divides, the daughter cells may be identical in every way, or they may change their patterns of gene expression to become functionally different from the parent cell.

Among prokaryotes and lower eukaryotes, the formation of spores is an example of such **cellular differentiation** (see Topic L3). Among complex multicellular eukaryotes, embryonic cells differentiate into highly specialized cells, for example muscle, nerve, liver and kidney. In all but a few exceptional cases, the DNA content remains the same, but the genes which are transcribed have changed. Differentiation is regulated by developmental control genes (see Topic N2). Mutations in these genes result in abnormal body plans, such as legs in the place of antennae in the fruit fly *Drosophila*. Studying such gene mutations allows the process of embryonic development to be understood. In multicellular organisms, co-ordination of the activities of the various tissues and organs is controlled by communication between them. This involves signaling molecules such as neurotransmitters, hormones and growth factors which are secreted by one tissue and act upon another through specific cell-surface receptors.

A2 SUBCELLULAR ORGANELLES

Key Notes

Nuclei

The membrane-bound nucleus contains the bulk of the cellular DNA in multiple chromosomes. Transcription of this DNA and processing of the RNA occurs here. Nucleoli are contained within the nucleus.

Mitochondria and chloroplasts

Mitochondria are the site of cellular respiration where nutrients are oxidized to CO_2 and water, and adenosine 5′-triphosphate (ATP) is generated. They are derived from prokaryotic symbionts and retain some DNA, RNA and protein synthetic machinery, though most of their proteins are encoded in the nucleus. Photosynthesis takes place in the chloroplasts of plants and eukaryotic algae. Chloroplasts have a basically similar structure to mitochondria but with a thylakoid membrane system containing the light-harvesting pigment chlorophyll.

Endoplasmic reticulum

The smooth endoplasmic reticulum is a cytoplasmic membrane system where many of the reactions of lipid biosynthesis and xenobiotic metabolism are carried out. The rough endoplasmic reticulum has attached ribosomes engaged in the synthesis of membrane-targeted and secreted proteins. These proteins are carried in vesicles to the Golgi complex for further processing and sorting.

Microbodies

The lysosomes contain degradative, hydrolytic enzymes; the peroxisomes contain enzymes which destroy certain potentially dangerous free radicals and hydrogen peroxide; the glyoxysomes of plants carry out the reactions of the glyoxylate cycle.

Organelle isolation

After disruption of the plasma membrane, the subcellular organelles can be separated from each other and purified by a combination of differential centrifugation and density gradient centrifugation (both rate zonal and isopycnic). Purity can be assayed by measuring organelle-specific enzymes.

Related topics

Cellular classification (A1)
rRNA processing and ribosomes (O1)
Translational control and post-translational events (Q4)

Nuclei

The eukaryotic nucleus carries the genetic information of the cell in multiple chromosomes, each containing a single DNA molecule (see Topics D2 and D3). The nucleus is bounded by a lipid double membrane, the nuclear envelope, containing pores which allow passage of moderately large molecules (see Topic A1, *Fig.* 2). Transcription of RNA takes place in the nucleus (see Section M) and the processed RNA molecules (see Section O) pass into the cytoplasm where translation takes place (see Section Q). **Nucleoli** are bodies within the nucleus

where rRNA is synthesized and ribosomes are partially assembled (see Topics M2 and O1).

Mitochondria and chloroplasts

Cellular respiration, that is the oxidation of nutrients to generate energy in the form of adenosine 5'-triphosphate (ATP), takes place in the **mitochondria**. These organelles are roughly 1–2 μm in diameter and there may be 1000–2000 per cell. They have a smooth outer membrane and a convoluted inner membrane that forms protrusions called **cristae** (see Topic A1, *Fig.* 2). They contain a small circular DNA molecule, mitochondrial-specific RNA and ribosomes on which some mitochondrial proteins are synthesized. However, the majority of mitochondrial (and chloroplast) proteins are encoded by nuclear DNA and synthesized in the cytoplasm. These latter proteins have specific **signal sequences** that target them to the mitochondria (see Topic Q4). The **chloroplasts** of plants are the site of photosynthesis, the light-dependent assimilation of CO_2 and water to form carbohydrates and oxygen. Though larger than mitochondria, they have a similar structure except that, in place of cristae, they have a third membrane system (the **thylakoids**) in the inner membrane space. These contain chlorophyll, which traps the light energy for photosynthesis. Chloroplasts are also partly genetically independent of the nucleus. Both mitochondria and chloroplasts are believed to have evolved from prokaryotes which had formed a symbiotic relationship with a primitive nucleated eukaryote.

Endoplasmic reticulum

The **endoplasmic reticulum** is an extensive membrane system within the cytoplasm and is continuous with the nuclear envelope (see Topic A1, *Fig.* 2). Two forms are visible in most cells. The **smooth** endoplasmic reticulum carries many membrane-bound enzymes, including those involved in the biosynthesis of certain lipids and the oxidation and detoxification of foreign compounds (**xenobiotics**) such as drugs. The **rough** endoplasmic reticulum (**RER**) is so-called because of the presence of many ribosomes. These ribosomes specifically synthesize proteins intended for secretion by the cell, such as plasma or milk proteins, or those destined for the plasma membrane or certain organelles. Apart from the plasma membrane proteins, which are initially incorporated into the RER membrane, these proteins are translocated into the interior space (**lumen**) of the RER where they are modified, often by **glycosylation** (see Topic Q4). The lipids and proteins synthesized on the RER are transported in specialized **transport vesicles** to the **Golgi complex**, a stack of flattened membrane vesicles which further modifies, sorts and directs them to their final destinations (see Topic A1, *Fig.* 2).

Microbodies

Lysosomes are small membrane-bound organelles which bud off from the Golgi complex and which contain a variety of digestive enzymes capable of degrading proteins, nucleic acids, lipids and carbohydrates. They act as recycling centers for macromolecules brought in from outside the cell or from damaged organelles. Some metabolic reactions which generate highly reactive free radicals and hydrogen peroxide are confined within organelles called **peroxisomes** to prevent these species from damaging cellular components. Peroxisomes contain the enzyme catalase, which destroys hydrogen peroxide:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Glyoxysomes are specialized plant peroxisomes which carry out the reactions of the glyoxylate cycle. Lysosomes, peroxisomes and glyoxysomes are collectively known as **microbodies**.

Organelle isolation

The plasma membrane of eukaryotes can be disrupted by various means including osmotic shock, controlled mechanical shear or by certain nonionic detergents. Organelles displaying large size and density differences, for example nuclei and mitochondria, can be separated from each other and from other organelles by ***differential centrifugation*** according to the value of their **sedimentation coefficients** (see Topic A4). The **cell lysate** is centrifuged at a speed which is high enough to sediment only the heaviest organelles, usually the nuclei. The supernatant containing all the other organelles is removed then centrifuged at a higher speed to sediment the mitochondria, and so on (*Fig. 1a*). This technique is also used to fractionate suspensions containing cell types of different sizes, for example red cells, white cells and platelets in blood. These crude preparations of cells, nuclei and mitochondria usually require further purification by **density gradient centrifugation**. This is also used to separate organelles of similar densities. In **rate zonal centrifugation**, the mixture is layered on top of a pre-formed concentration (and, therefore, density) gradient of a suitable medium in a centrifuge tube. Upon centrifugation, bands or zones of the different components sediment at different rates depending on their sedimentation coefficients, and separate (*Fig. 1b*). The purpose of the density gradient of the supporting medium is to prevent convective mixing of the components after separation (i.e. to provide stability) and to ensure linear sedimentation rates of the components (it compensates for the acceleration of the components as they move further down the tube). In **equilibrium (isopycnic) centrifugation**, the density gradient extends to a density higher than that of one or more components of the mixture so that these components come to equilibrium at a point equal to their own density and stop moving. In this case, the density gradient can either be pre-formed, and the sample layered on top, or self-forming, in which case the sample may be mixed with the gradient material (*Fig. 1c*). Density gradients are made from substances such as sucrose, Ficoll (a synthetic polysaccharide), metrizamide (a synthetic iodinated heavy compound) or cesium chloride (CsCl), for separation of nucleic acids (see Topics C2 and G2). Purity of the subcellular fraction can be determined using an electron microscope or by assaying enzyme activities known to be associated specifically with particular organelles, for example succinate dehydrogenase in mitochondria.

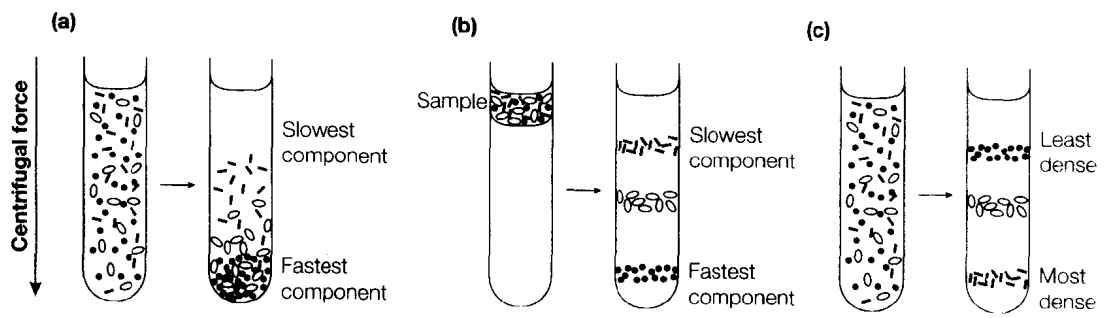

Fig. 1. Centrifugation techniques. (a) Differential, (b) rate zonal and (c) isopycnic (equilibrium).