

Edited by Michael Wink

An Introduction to Molecular Biotechnology

Fundamentals, Methods and Applications

Third, Completely Revised Edition



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Editor

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Abbreviations

1 Å	=0.1 nm	ATP	adenosine triphosphate
aa-tRNA	aminoacyl-tRNA	att	attachment site
AAV	adeno-associated virus	BAC	bacterial artificial chromosome
ABC	ATP-binding cassette	bcl2	B-cell leukemia lymphoma 2 (protein protecting against apoptosis)
Acetyl CoA	acetyl coenzyme A	BfArM	German Bundesinstitut für Arzneimittel und Medizinprodukte
AcNPV	<i>Autographa californica</i> nuclear polyhedrosis virus	β-Gal	β-galactosidase
ACRS	amplification-created restriction sites	BHK-21	baby hamster kidney cells
ACTH	adrenocorticotrophic hormone	BLA	biologics licence application
ADA	adenosine deaminase	BLAST	Basic Local Alignment Search Tool
ADEPT	antibody-directed enzyme prodrug therapy	BMP	bone morphogenetic proteins
ADME-T	absorption, distribution, metabolism, excretion, and toxicity	bp	base pairs
ADP	adenosine diphosphate	BrdU	bromodeoxyuridine
ADRs	adverse drug reactions	CA	correspondence analysis
AEC	aminoethylcysteine	CAD	coronary artery disease
AFLP	amplified fragment length polymorphism	CaM-Kinase	Ca ²⁺ /calmodulin-dependent protein kinase
AFM	atomic force microscope	cAMP	cyclic AMP
AIDS	acquired immunodeficiency syndrome	cap	AAV gene mediating encapsulation
ALS	amyotrophic lateral sclerosis	CARS	coherent anti-Raman scattering
AMP	adenosine monophosphate	CAT	Committee for Advanced Therapies
AMPA	α-amino-3-hydroxyl- 5-methyl-4-isoxazol-propionate	CBER	Center for Biologics Evaluation and Research
Amp ^r	ampicillin resistance gene	CC	chromatin remodeling complex
AMV	avian myeloblastosis virus	CCD	charge-coupled device
ANN	artificial neural network	CDER	Center for Drug Evaluation and Research
AO	acridine orange	CDK	cyclin-dependent kinase
AOX1	alcohol oxidase 1	cDNA	copy DNA
APC	anaphase-promoting complex	CDR	complementarity-determining region
ApoB100	apolipoprotein B100	CDRH	Center for Devices and Radiological Health
ApoE	apolipoprotein E	CEO	chief executive officer
APP	amyloid precursor protein	CFP	cyan fluorescent protein
ARMS	amplification refractory mutation system		
ARS	autonomously replicating sequence		

CFTR	cystic fibrosis transmembrane regulator	Dox	doxycycline
CGAP	Cancer Genome Anatomy Project	ds diabodies	disulfide-stabilized diabodies
CGH	comparative genome hybridization	dsDNA	double-stranded DNA
CHMP	Committee for Medicinal Products for Human Use	dsFv-fragment	disulfide-stabilized Fv fragment
CHO	Chinese hamster ovary	dsRNA	double-stranded RNA
CIP	calf intestinal phosphatase	DtxR	diphtheria toxin repressor
CML	chronic myeloid leukemia	Ebola-Z	envelope protein of the Ebola-Zaire virus, which has a high affinity to lung epithelial cells
CMN	<i>Corynebacterium–Mycobacterium–Nocardia</i> group	EC ₅₀	effective concentration, the dose or concentration that produces a 50% effect in the test population within a specified time
CaMV	cauliflower mosaic virus	ECD	electron capture dissociation
CMV	cytomegalovirus	EDTA	ethylenediaminetetraacetic acid
CNS	central nervous system	ee	enantiomeric excess
COMP	Committee for Orphan Medicinal Products	EF2	elongation factor 2
COS-1	simian cell line, CV-1, transformed by origin-defective mutant of SV40	EF-Tu	elongation factor Tu
cpDNA	chloroplast DNA	EGF	epidermal growth factor
CPMV	cowpea mosaic virus	EGFP	enhanced green fluorescent protein
cPPT-sequence	central polypurine tract – regulatory element in lentiviral vectors that facilitates double strand synthesis and the nuclear import of the pre-integration complex	EGTA	ethylene glycol bis(2-aminoethyl)tetraacetic acid
CSF	colony-stimulating factor	EIAV	equine infectious anemia virus
CSO	contract service organization	ELISA	enzyme-linked immunosorbent assay
CTAB	cetyltrimethylammonium bromide	EM	electron microscope
CVM	Center for Veterinary Medicine	EMA	European Medicines Agency
CVMP	Committee for Medicinal Products for Veterinary Use	EMBL	European Molecular Biology Laboratory
2D	two-dimensional	EMCV	encephalomyocarditis virus
Da	Dalton	EMSA	electrophoretic mobility shift assay
DAG	diacylglycerol	EMEA	European Agency for the Evaluation of Medicinal Products
DAPI	4,6-diamidino-2-phenylindole	ENU	<i>N</i> -ethyl- <i>N</i> -nitrosourea
dATP	deoxyadenosine triphosphate	env	retroviral gene coding for viral envelope proteins
DBD	DNA-binding domain	EPO	European Patent Office
DAC	divide-and-conquer strategy	EPR effect	enhanced permeability and retention effect
DD	differential display	EPC	European Patent Convention
DDBJ	DNA Data Bank of Japan	ER	endoplasmic reticulum
ddNTP	dideoxynucleotide triphosphate	ESI	electrospray ionization
DEAE	diethylaminoethyl	EST	expressed sequence tags
dHPLC	denaturing HPLC	ES cells	embryonic stem cells
DIC	differential interference contrast	EtBr	ethidium bromide
DIP	Database of Interacting Proteins	Fab-fragment	antigen-binding fragment
DNA	deoxyribonucleic acid	FACS	fluorescence-activated cell sorter
DNAse	deoxyribonuclease		
dNTP	deoxynucleoside triphosphate		

FAD	flavin adenine dinucleotide	GTC	guanidinium isothiocyanate
FBA	flux balance analysis	GTP	guanosine triphosphate
FCS	fluorescence correlation spectroscopy	GUS	glucuronidase
FDA	Food and Drug Administration	GMO	genetically modified organism
FFL	feed-forward loop	HA	hemagglutinin
FGF	fibroblast growth factor	HCM	hypertrophic cardiomyopathy
FISH	fluorescence <i>in situ</i> hybridization	HCV	hepatitis C virus
FIV	feline immunodeficiency virus	HEK	human embryonic kidney
FKBP	FK506-binding protein	HeLa cells	human cancer cell line (isolated from donor Helene Larsen)
FLIM	fluorescence lifetime imaging microscopy	HER 2	human epidermal growth factor 2
FLIPR	fluorescent imaging plate reader	HGH	human growth hormone
FMN	flavin mononucleotide	HIC	hydrophobic interaction chromatography
FPLC	fast performance liquid chromatography	His ₆	hexahistidine tag
FRAP	fluorescence recovery after photobleaching	HIV	human immunodeficiency virus, a retrovirus
FRET	fluorescence resonance energy transfer	HIV 1	human immunodeficiency virus 1
FT-ICR	Fourier transformation cyclotron resonance, method in mass spectroscopy	HLA	human leukocyte antigen
FtsZ	prokaryotic cell division protein	hnRNA	heterogeneous nuclear RNA
Fur	ferric uptake regulator	HPLC	high-performance liquid chromatography
Fv-fragment	variable fragment	HPT	hygromycin phosphotransferase
FWHM	full width at half maximum	HPV	human papillomavirus
GABA	gamma-aminobutyric acid	HSP	high-scoring segment pairs
Gag	retroviral gene coding for structural proteins	HSP	heat shock protein
Gal	galactose	HSV-1	herpes simplex virus
GAP	GTPase-activating protein	HTS	high-throughput analysis
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	HUGO	Human Genome Organization
Gb	gigabases	HV	herpesvirus
GCC	German cDNA consortium	IAS	international accounting standard
GCG	Genetics Computer Group	ICDH	isocitric dehydrogenase
GCP	good clinical practice	ICH	International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ΔG_d	free enthalpy	ICL	isocitric lyase
GDH	glutamate dehydrogenase	ICP-MS	inductively coupled plasma mass spectrometry
GDP	guanosine diphosphate	ICR-MS	ion cyclotron resonance mass spectrometer
GEF	guanine exchange factor	IDA	iminodiacetic acid
GEO	gene expression omnibus	IEF	isoelectric focusing
GFP	green fluorescent protein	Ig	immunoglobulin
GM-CSF	granulocyte/macrophage colony-stimulating factor	IHF	integration host factor
GO	Gene Ontology	IMAC	immobilized metal affinity chromatography
GOI	gene of interest	IND-Status	investigational new drug status
GPCR	G-protein-coupled receptor	IP ₃	inositol-1,4,5-triphosphate
GPI anchor	glycosylphosphatidylinositol anchor	IPO	initial public offering
GRAS	generally regarded as safe	IPTG	isopropyl- β -D-thiogalactoside
GST	glutathione-S-transferase		

IRs	inverted repeats	MAGE-ML	microarray gene expression
IR	investor relations		markup language
IRES	internal ribosome entry site	MALDI	matrix-assisted laser
ISAAA	International Service for the Acquisition of Agri-biotech Applications	6-MAM	desorption/ionization 6-monoacetylmorphine
ISH	<i>in situ</i> hybridization	MAP	microtubule-associated protein
ISSR	inter-simple sequence repeats	MAP	mitosis-activating protein
ITC	isothermal titration calorimetry	Mb	megabases
ITR	inverse terminal repeats – regulatory elements in adenoviruses and AAV	MBP	maltose-binding protein
i.v.	intravenous	MCS	multiple cloning site
k_a	second-order velocity constant in bimolecular association	M-CSF	macrophage colony-stimulating factor
Kan ^r	kanamycin resistance gene	MDR	multidrug resistance protein
K_{av}	specific distribution coefficient	MDS	multidimensional scaling
Kb	kilobases	MGC	Mammalian Gene Collection
k_d	first-order velocity constant in unimolecular dissociation	MHC	major histocompatibility complex
$K_d = k_d/k_a$	velocity constant in dissociation/ K_a in association	MIAME	minimum information about a microarray experiment
KDa	kilodalton	miRNA	microRNA
KDEL	amino acid sequence for proteins remaining in the ER	MIT	Massachusetts Institute of Technology
KDR receptor	kinase insert domain-containing receptor	MoMLV	Moloney murine leukemia virus
KEGG	Kyoto Encyclopedia of Genes and Genomes	Mowse	molecular weight search
Lac	lactose	MPF	M-phase promotion factor
LASER	light amplification by stimulated emission of radiation	MPSS	Massively Parallel Signature Sequencing
LB	left border	Mreb/Mbl	proteins of prokaryotic cytoskeleton
LB	Luria-Bertani medium	mRNA	messenger RNA
LCR	ligation chain reaction	MRSA	methicillin-resistant <i>Staphylococcus aureus</i>
LDL	low-density lipoprotein	MS	mass spectrometry
LIMS	laboratory information management systems	MSG	monosodium glutamate
LINE	long interspersed elements	MS-PCR	mutationally separated PCR
LSC	laser scanning cytometer	MTA	material transfer agreement
LTQ	linear trap quadrupole	mtDNA	mitochondrial DNA
LTQ-FT-ICR	linear trap quadrupole–Fourier transformation ion cyclotron resonance	MULVR	Moloney murine leukemia virus
LTR	long terminal repeats; regulatory elements in retroviruses	MW	molecular weight
LUMIER	LUMInescence-based Mammalian intERactome	μ F	μ Farad
MAC	mammalian artificial chromosome	nAChR	nicotinic acetylcholine receptor
mAChR	muscarinic acetylcholine receptor	NAD	nicotinamide adenine dinucleotide
		NAPPA	nucleic acid programmable protein array
		NCBI	National Center for Biotechnology Information
		NDA	new drug application
		NDP	nucleoside diphosphate
		NDPK	nucleoside diphosphates kinase
		NFjB	nuclear factor jB
		NIH	National Institutes of Health
		NK cell	natural killer cell
		NMDA receptor	<i>N</i> -methyl-D-aspartate receptor

NMR	nuclear magnetic resonance	RAPD	random amplification of
NPTII	neomycin phosphotransferase II		polymorphic DNA
NSAID	nonsteroidal anti-inflammatory drug	RAP-PCR	RNA arbitrarily primed PCR
		RB	right border
NTA	nitrilotriacetic acid	RBD	RNA-binding domain
NTP	nucleoside triphosphate	Rb gene	retinoblastoma gene
OD	optical density	RBS	ribosome-binding site
ODE	ordinary differential equation	RDA	representative difference analysis
ODHC	2-oxoglutarate dehydrogenase		
OMIM	Online Mendelian Inheritance in Man	RdRp	RNA-dependent RNA polymerase
ORF	open reading frame	rep	AAV gene mediating replication
ori	origin of replication	RES	reticuloendothelial system
OXA complex	membrane translocator in mitochondria	RFLP	restriction fragment length polymorphism
PAC	P1-derived artificial chromosome	R_f -value	retention factor
PAGE	polyacrylamide gel electrophoresis	RGS	regulator of G-protein signaling
PAZ domain	<i>PIWI/Argonaute/Zwille domain</i>	RISC	RNA-induced silencing complex
PCA	principal component analysis	RNA	ribonucleic acid
PCR	polymerase chain reaction	RNAi	RNA interference
PDB	protein data bank	RNP	ribonucleoprotein
PEG	polyethylene glycol	rpm	revolutions per minute
PFAM	protein families database of alignments and HMMs	RRE	regulatory element in a lentiviral vector, enhancing the nuclear export of viral RNA
PFG	pulsed-field gel electrophoresis	rRNA	ribosomal RNA
PI	propidium iodide	RSV	respiratory syncytial virus
PIR	protein information resource	RSV	promoter of the Rous sarcoma virus
piRNA	piwi-interacting RNA	RT	reverse transcriptase
PKA	protein kinase A	rtTA	tetracycline-sensitive regulatory unit
PKC	protein kinase C		
PK data	pharmacokinetic data	SAGE	serial analysis of gene expression
PLoS	Public Library of Science	SALM	spectrally assigned localization microscopy
PMSF	phenylmethylsulfonyl fluoride		
PNA	peptide nucleic acid	SAM	S-adenosylmethionine
PNGaseF	peptide <i>N</i> -glycosidase F	sc diabodies	single-chain diabodies
PNK	T4 polynucleotide kinase	scFab	single-chain Fab fragment
pol	retroviral gene coding for reverse transcriptase and integrase	scFv/sFv fragment	single-chain Fv fragment
		SCID	severe combined immunodeficiency
P_{PH}	polyhedrin promoter		
PR	public relations	SCOP	structural classification of proteins
psi	retroviral packaging signal		
PTGS	posttranscriptional gene silencing	SDS	sodium dodecyl sulfate
		SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
PTI	pancreatic trypsin inhibitor		
Q-FT-ICR	q-Fourier transform ion cyclotron resonance	SELEX	systematic evolution of ligands by exponential enrichment
Q-TOF	quadrupole time-of-flight		
RACE	rapid amplification of cDNA ends	SEM	scanning electron microscope
		Sf cells	<i>Spodoptera frugiperda</i> cells
Ran	protein involved in nuclear import	SFM	scanning force microscope
		SFV	Semliki Forest virus

SH1	Src homology domain 1 = kinase domain	TIM	translocase of inner membrane
SH2	Src homology domain 2	T_m	melting temperature of dsDNA
SH3	Src homology domain 3	TNF	tumor necrosis factor
SHG	second harmonic generation	TOF	time of flight
SIM	single input	TOM	translocase of outer membrane
SIN	self-inactivating lentiviral vectors, due to a 3' LTR mutation	t-PA	tissue plasminogen activator
SINE	scattered or short interspersed elements	TRE	tetracycline-responsive element
siRNA	small interfering RNA	TRIPs	Trade-Related Aspects of Intellectual Property Rights
SIV	simian immunodeficiency virus	tRNA	transfer RNA
SNARE proteins	SNAP receptor proteins	Trp	tryptophan
SNP	single nucleotide polymorphism	t-SNARE	protein in target membrane to which v-SNARE binds
snRNA	small nuclear RNA	TSS	transformation and storage solution
snRNP	small nuclear ribonucleoprotein	tTA	tetracycline-controlled transactivator
SOP	stock option program	TY	transposon from yeast
SP function	sum-of-pairs function	UPOV	Union for the Protection of New Varieties of Plants
SPA	scintillation proximity assay	US-GAAP	US generally accepted accounting principle
SPDM	spectral precision distance microscopy	UV	ultraviolet
SPF	S-phase promotion factor	V_0	empty volume
SRP	signal recognition particle	VC	venture capital
SSB	single-strand binding proteins	V_e	elution volume
SSCP	single-strand conformation polymorphism	VEGF	vascular endothelial growth factor
ssDNA	single-stranded DNA	VIP	vasoactive peptide
SSH	suppression subtractive hybridization	VNTR	variable number tandem repeats
SssI methylase	methylase from <i>Spiroplasma</i>	v-SNARE	protein in vesicular membrane, binding to t-SNARE
ssRNA	single-stranded RNA	VSV-G	envelope protein of vesicular stomatitis virus, great affinity to a wide range of cells
STED	stimulated emission depletion	V_t	total volume
STEM	scanning transmission electron microscope	wNAPPA	modified nucleic acid programmable protein array
stRNA	small temporal RNA	WPRE	woodchuck hepatitis virus posttranscriptional regulatory element
STS	sequence-tagged site	X-Gal	5-bromo-4-chloro-3-indolyl- β - D-galactopyranoside
SV40	Simian virus type 40	YAC	yeast artificial chromosome
TBP	TATA-binding protein	YEpl	yeast episomal plasmid
T_c	cytotoxic T cells	YFP	yellow fluorescence protein
Tc	tetracycline	YIp	yeast-integrating plasmid
T-DNA	transfer DNA	YRp	yeast-replicating plasmid
TEM	transmission electron microscope	Yth	yeast two-hybrid
TEV	tobacco etch virus		
T_H	T helper cell		
THG	third harmonic generation		
TIGR	The Institute for Genome Research		

Part I

Fundamentals of Cellular and Molecular Biology

1

The Cell as the Basic Unit of Life

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The base unit of life is the **cell**. Cells constitute the base element of all **prokaryotic cells** (cells without a cell nucleus, e.g. **Bacteria** and **Archaea**) and **eukaryotic cells** (or **Eukarya**) (cells possessing a nucleus, e.g. protozoa, fungi, plants, and animals). Cells are small, membrane-bound units with a diameter of 1–20 μm and are filled with concentrated aqueous solutions. Cells are not created *de novo*, but possess the ability to copy themselves, meaning that they emerge from the division of a previous cell. This means that all cells, since the beginning of life (around 4 billion years ago), are connected with each other in a continuous lineage. In 1885, the famous cell biologist Rudolf Virchow conceived the law of *omnis cellula e cellula* (all cells arise from cells), which is still valid today.

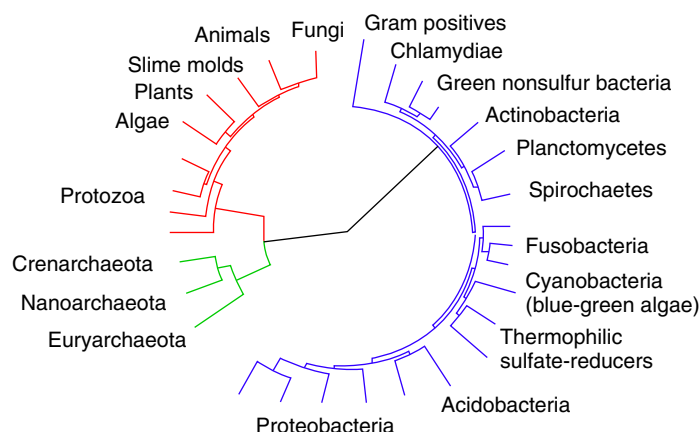
The structure and composition of all cells are very similar due to their shared evolution and phylogeny (Figure 1.1). We see an astonishing constancy in fundamental structures and mechanisms. Owing to this, it is possible to limit the discussion of the general characteristics of a cell to a few basic types (Figure 1.2):

- Plant cells
- Animal cells

Nucleotide sequences from 16S rRNA, amino acid sequences of cytoskeleton proteins, and characteristics of the cell structure were used to reconstruct this phylogenetic tree. Prokaryotes are divided into **Bacteria** and **Archaea**. Archaea form a sister group with eukaryotes; they share important characteristics (Tables 1.1 and 1.2). Many monophyletic groups can be recognized within the eukaryotes (diplomonads/trichomonads, Euglenozoa, Alveolata, Stramenopilata [heterokonts], red algae and green algae/plants, fungi and animals; see Tables 6.3–6.5 for details).

A highly resolved tree of life is based on completely sequenced genomes (Ciccarelli 2006). The image was generated using Interactive Tree Of Life (iTOL) (Letunic 2007), an online phylogenetic tree viewer and Tree of Life resource. Eukaryotes are colored red, archaea green, and bacteria blue.

Figure 1.1 Tree of life – phylogeny of life domains.



- Bacterial cells

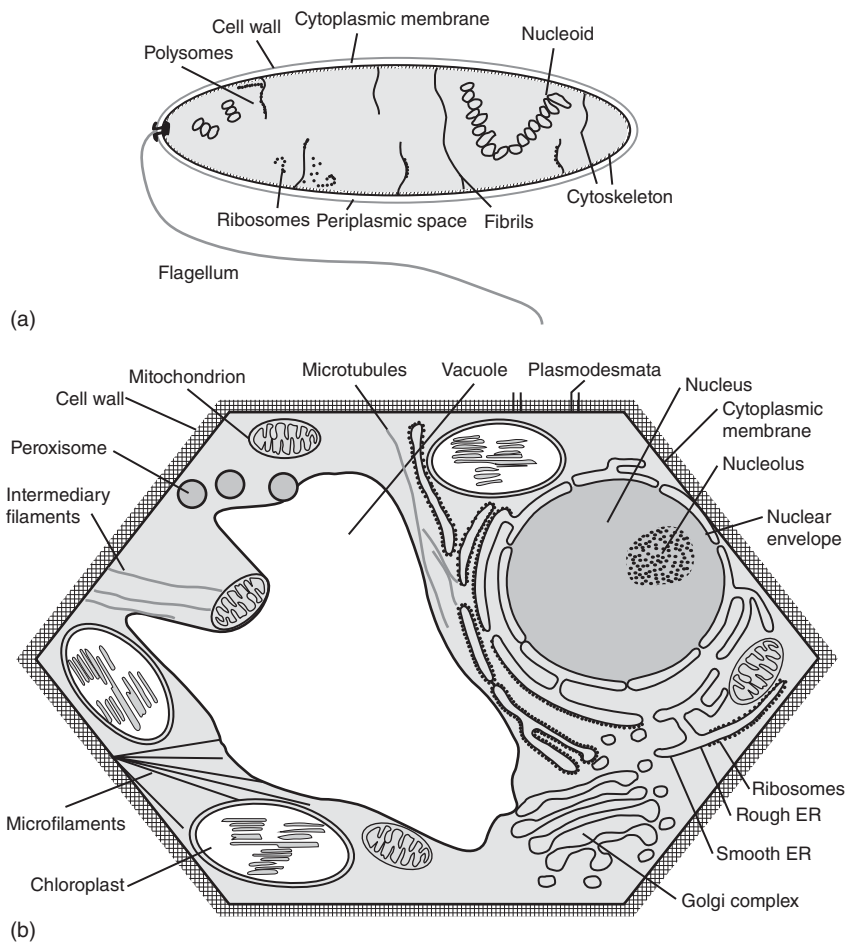


Figure 1.2 Schematic structure of prokaryotic and eukaryotic cells. (a) Bacterial cell, (b) plant mesophyll cell, and (c) animal cell.

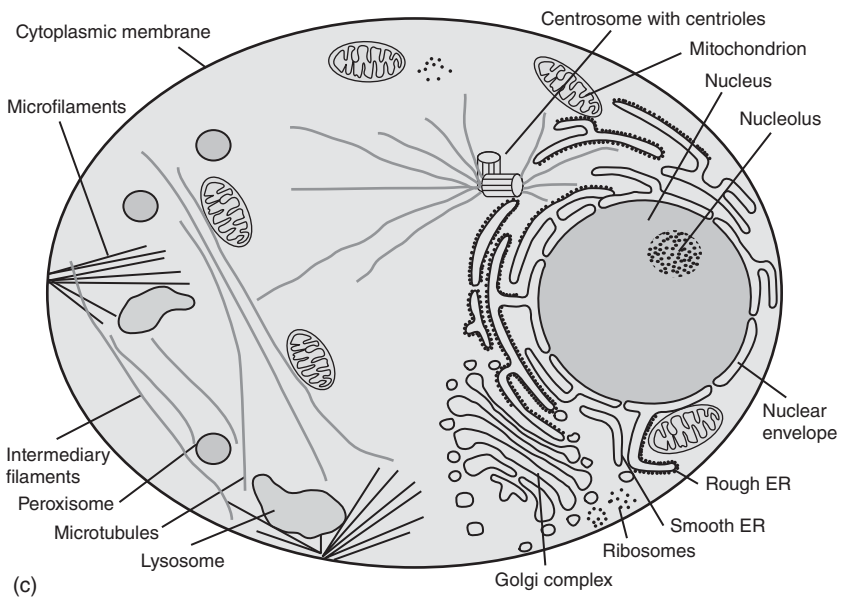


Figure 1.2 (Continued)