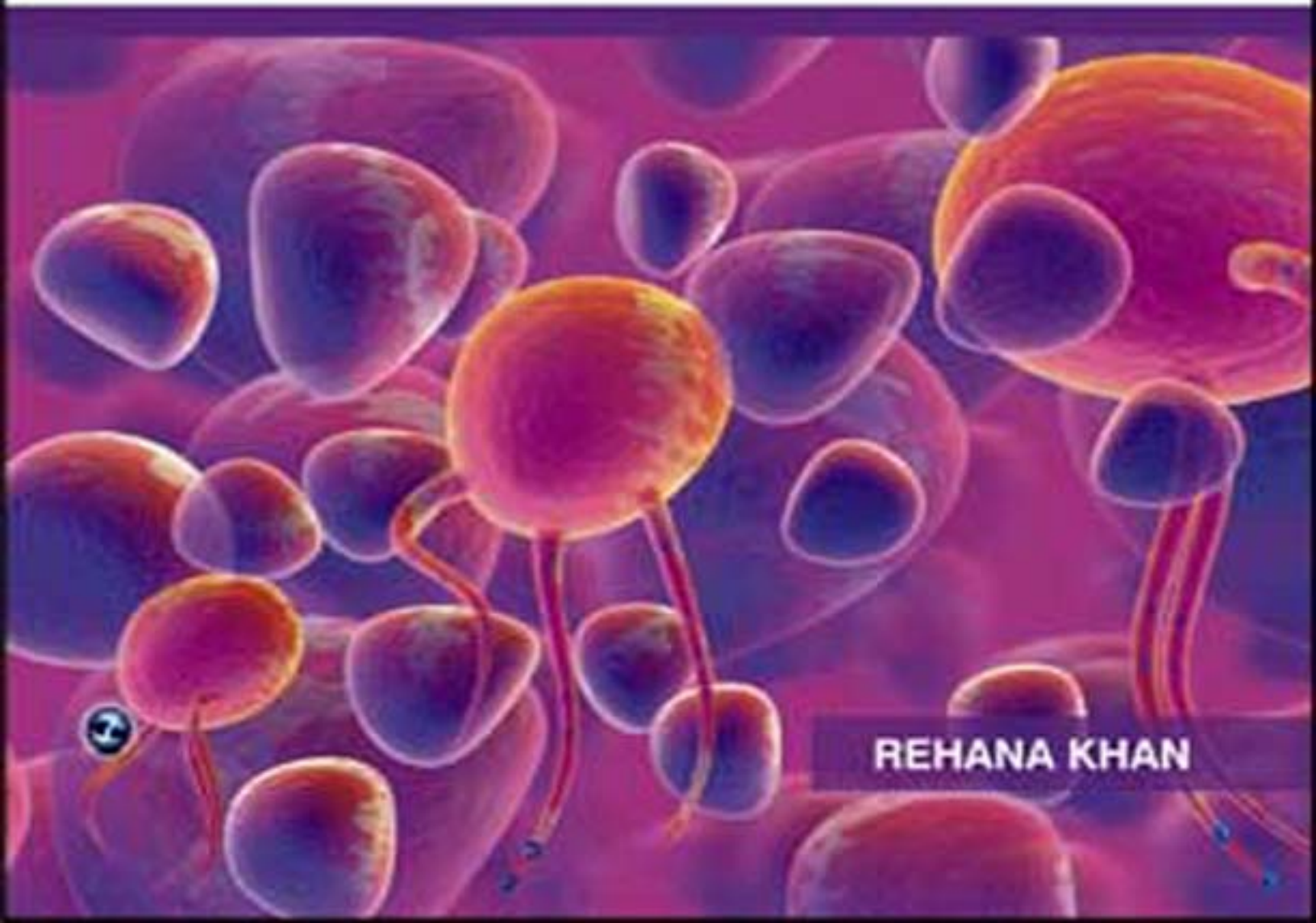
SECOND EDITION

A Textbook of

BIOTECHNOLOGY

Volume-I
Genetics and Molecular Biology



REHANA KHAN

A TEXTBOOK OF
BIOTECHNOLOGY

VOLUME I: GENETICS AND MOLECULAR BIOLOGY

By

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Dedicated
to
My Family
&
My Teacher

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PREFACE FOR SECOND EDITION

Much knowledge and skill have gathered momentum in biotechnology. After the publication of first edition of this book, new equipments, tools and techniques have added to usefulness for common man.

Government patronage and conducive policy for promoting, teaching, training and popularizing the benefits for the common man has helped a lot. The new generation find new occupation and carrier opportunities in biotechnology by which the fruit of well being will be shared at national and international level.

Though first edition got a welcome response by the beneficiaries still the stake hilder are expecting more with some edition to equipped them with new techniques in researches, to make them multiskilled and to take additional responsibilities with new vision.

The author express gratitude for the teachers, students and researchers dealing biotechnological problems who sent their valuable suggestions and prompted me to update and make it more useful and value added in its worth.

—AUTHOR

PREFACE FOR FIRST EDITION

The aim of this book is to present the subject of Biotechnology to the undergraduate B.Sc. IInd Year students of Biotechnology, M.P. Universities and students of various other Universities. This book provides an accurate and understandable matter on Biotechnology.

The author has been extra cautious in presenting the matter in easy and lucid language. The diagrams are especially designed for clarity and simplicity. It is comparable at international level and fully based on scientific research and teaching. Thus, in this book many topics which are not easily available have been incorporated for the convenience of the students.

I am very thankful to our principal Prof. Shakoor Khan for his precious suggestion, support and help.

I am very much thankful to my teacher Dr. Rabia Sultan, Prof. and Head, Saifia Science College, who inspired and guided me to bring this book in a readable form.

We all express our gratitude for the remarkable work of typing that has been done by Mr. Mirza Arif Baig.

We shall welcome comments and criticism from readers.

—AUTHOR

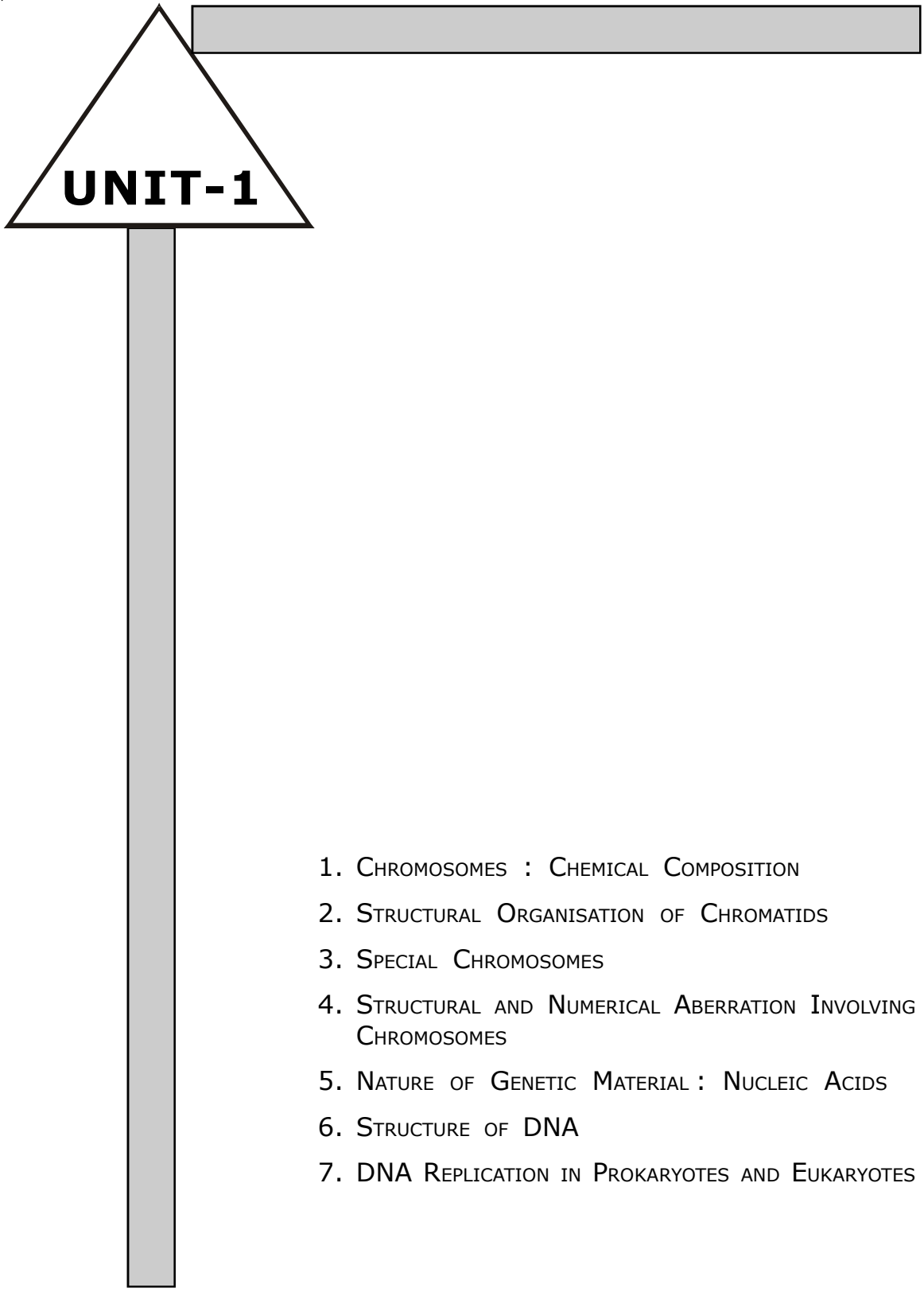
ACKNOWLEDGEMENT

The completion of the book has not at all been an easy task. It has been completed with the co-operation of so many hands. Hence, I acknowledge it heartily.

First and foremost I am thankful to my colleague Huda Zafar, who is Assistant Professor in the Department of Zoology and my students—Farheen, Versha, Avrti and M.A. Khan for the time and energy they have devoted in this job.

I cannot forget the co-operation and inspiration of my mother, my husband, Mr. Firdaus Khan without which it would have never been a success.

—AUTHOR



1. Chromosomes : Chemical Composition
2. Structural Organisation of Chromatids
3. Special Chromosomes
4. Structural and Numerical Aberration Involving Chromosomes
5. Nature of Genetic Material : Nucleic Acids
6. Structure of DNA
7. DNA Replication in Prokaryotes and Eukaryotes

1 CHROMOSOMES: CHEMICAL COMPOSITION

INTRODUCTION

Chromosomes are the nuclear components of special organization, individuality and function.

They have the capability of self reproduction and play a vital role in the process of heredity, mutation, variation and evolutionary development.

It was *E. Strasburger* (1875), who observed thread-like structures during cell division. These thread-like structures were named as chromosomes (Gr. *Chrom* — colour, *soma* — body) by *Waldeyer* in 1888.

The dark staining network, which can readily be stained with basic dyes is termed as nuclear reticulum. The threads of this reticulum are made up of chromatin, which can be seen in the interphase nuclear stage during cell division. These chromatin threads or ribbon like structures are called CHROMOSOMES.

CHEMICAL COMPOSITION OF CHROMOSOMES

With the development of technology in biochemistry, the chemical composition of chromosomes has been carried out which revealed that major components of chromosomes

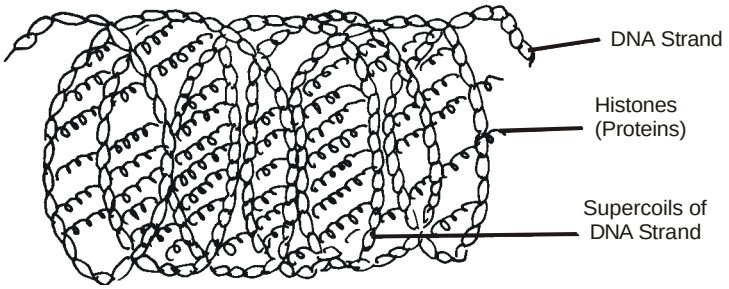


Fig. 1.1. Depicting possible relationship of histone protein and DNA double helix and chromonemata.

are DNA (Deoxyribose Nucleic Acid), RNA (Ribo Nucleic Acid), histones (basic proteins of low molecular weights) and acidic proteins (non histone proteins). Calcium has also been found associated in addition to these constituents but the exact way in which the nucleoprotein complex is assembled is still not very clear. It is thought by the cytologists

that RNA is localised at certain regions of the chromosome but proteins are associated with DNA molecule along its entire length. RNA seems to be associated with DNA because some part or the other of chromatin is always being transcribed. Therefore, RNA is the transcription product of chromatin and probably not its integral part. According to *Ambrose & Easty* (1970), the electrical properties of nucleoprotein mainly determine the stability of chromosomes. Histones partly neutralize the negatively charged phosphate groups of DNA. Further neutralization is brought about by calcium bridges formed by the Ca^{++} ion between phosphate groups. On the basis of *Ambrose & Gopal Ayenger's* (1952) observations it has also been reported that any factor which reduces the negative charges on nucleoprotein will tend to lead to the formation of a more compact structure and conversely any factor, which increases the negative charges will cause repulsive forces to operate, thus expanding the chromosomes.

Table 1.1. Showing Variation in Chemical Composition of Chromatin from the Same Source

Source		*% DNA*	*% RNA*	*% Histone*	*% Non-histone*
Pea	Embryonic axis	39	10	40	11
	Vegetable bud	40	4	52	4
	Growing cotyledon	43.5	6	34.5	16

The relative proportion of components suggests that, generally, there occurs more protein than DNA in chromosomes. Relative amount of different constituents of chromosome are given as follows:

DNA-histone complex	90 – 92%
Histone	55%
DNA	45%
Residual parts (protein) of chromosomes	8 – 10%
DNA	2 – 3%
RNA	12 – 14%
Acid proteins or protomers	83 – 86%

1. DNA
2. Histone protein
3. Non-histone protein

1. DNA

DNA is the most important chemical component of chromatin, since it plays the central role of controlling heredity. The most convenient of DNA is picogram (10^{-12}). DNA of chromatin represents the following two phenomena:

The C-value. The DNA in nuclei was stained using the feulgen reaction and the amount of stain in single nuclei was measured using a special microscope called cytophotometer. Both of these techniques demonstrated that nuclei contain a constant amount of DNA. Thus, all the cells in an organism contain the same DNA content (2C) provided that they are diploid. Gametes are haploid and, therefore, have half the DNA content (1C). Some tissues such as liver, contain occasional cells that are

polyploid and their nuclei have a correspondingly higher DNA content (4C or 8C) (see Table 1.2).

Table 1.2. DNA Content and Chromosome Component (after *De Robertis* and *De Robertis Jr.*, 1987)

S.No.	*Cells*	*Mean DNA-feulgen Content*	*Pressured Chromosome Set*
1.	Spermatid	1.68 (1C)	Haploid (*n*)
2.	Liver	3.16 (2C)	Diploid (2*n*)
3.	Liver	6.30 (4C)	Tetraploid (4*n*)
4.	Liver	12.80 (8C)	Octaploid (8*n*)

Thus, each species has a characteristic content of DNA, which is constant in all individuals of that species. This is called the C-value.

The C-value Paradox. Eukaryotes vary greatly in DNA content but always contain much more DNA than prokaryotes. Lower eukaryotes in general have less DNA, such as nematode *Caenorhabditis elegans* which has only 20 times more DNA than *E. coli*, or the fruit fly (*D. melanogaster*) which has 40 times more DNA (*i.e.,* 0.18 pg or picogram per haploid genome). Vertebrates have greater DNA content (about 3 pg), in general about 700 times more than *E. coli.* One of the highest DNA content is that of the *Salamander amphivma* which has 84 pg of DNA. Man has about 3 pg of DNA genome, or 3×10^9 base pairs, *i.e.,* the human genome could accommodate about 3 million average sized proteins if all the DNA were coding (or containing structural genes) and if this was true, salamanders would have 30 times more genes than human beings. This is called C-value paradox (*Gall,* 1981). It was detected quite early that there was little connection between the morphological complexity of eukaryotic organisms and their DNA content. For example, *E. coli* (containing 3,400,000 base pairs in its DNA) has about 3000 genes. Although it is difficult to estimate how many different genes exist in the human genome, there are probably not more than 20000 to 30000 genes. There is no reason to believe that salamanders should have more.

(**Note:** *According to a most recent estimate, there are 100000 genes in human genome, see Deviah, 1994*).

2. Histones

Histones are very basic proteins, basic, because they are enriched in the amino acids—arginine and lysine, to a level of about 24 mole per cent. Arginine and lysine at physiological pH are cationic and can interact electrostatically with anionic nucleic acids. Thus, being basic, histones bind tightly to DNA which is an acid. There are five types of histones in the eukaryotic chromosomes, namely H1, H2A, H2B, H3 and H4.

One of the important discoveries that has come from chemical studies is that the primary structures of histones have been highly conserved during evolutionary history. For example, histone H4 of calf and of garden pea contains only two amino acid differences in a protein of 102 residues (*DeLange*, 1969). Likewise, the sequence of histone H3 from rat differs only in two amino acids from that of pea, out of 102 total amino acid residues. These organisms are estimated to have an evolutionary history of at least 600 million years, during which they diverged structurally. This conservation of structure suggests that over the eras, histones have a very similar and crucial role in maintaining the structural and functional integrity of chromatin. Such an evolutionary conservation

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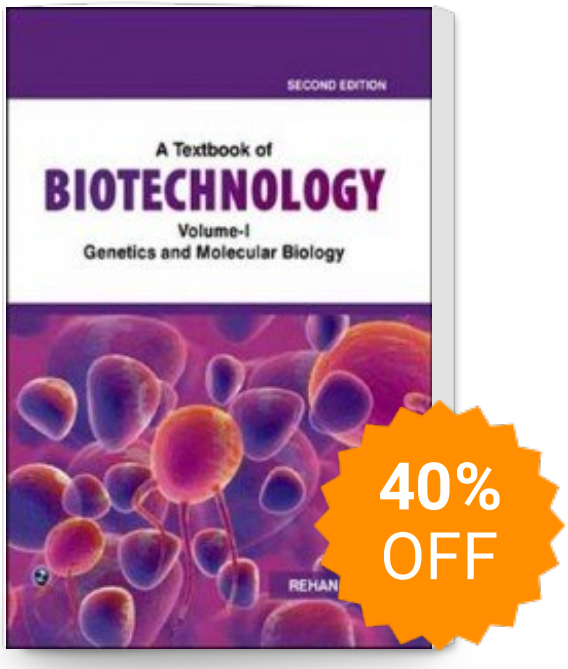

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