DNA, Chromatin and Chromosomes

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Preface

This book is chiefly about chromatin, which is the packaged form of the genetic material found in all higher organisms. Knowledge of chromatin, its structure, replication and activity, has increased greatly in recent years, and the book endeavours to provide a reasonably up to date account of present understanding in this area for both undergraduate and postgraduate students. Within a living cell the crucial element of chromatin is of course the DNA. In addition, for certain critical stages in the life of the cell, the chromatin itself is organized into discrete blocks, the chromosomes. It follows that these three aspects of genetic organization are inevitably bound up together, and chromatin cannot be usefully or seriously discussed without repeated reference to the biochemistry of DNA on the one hand, and the biology of chromosomes on the other. Thus this book emerges as one devoted to a unified triad of topics, DNA, chromatin and chromosomes, but with a central emphasis on the middle element.

Our plan in writing the book has been to combine our varied interests in DNA, chromatin and gene expression to form a relatively coherent account which no one of us could have undertaken on our own. The task has been rendered more exciting but less straightforward by the current fast rate of change in the field of molecular genetics, and the ground has more than once shifted under our feet while the book was being written. Nevertheless, it has proved an interesting exercise for us and we hope that some of the stimulation that we have found in the rapid progress of the subject will be carried over to those who read and use the book.

Many of our friends and colleagues have been of great assistance in its preparation, especially Dr. M. Ashburner, Professor M. Callan, Dr. T. Drabble, Dr. S. Gregory, Dr. V. Hilder, Dr. A. MacGillivray and Professor H. MacGregor. We also owe a debt of gratitude to Robert Campbell and the staff at Blackwell Scientific Publications who have patiently collaborated in the production of this book.

Summary of Chapters

CHAPTER 1 COMPONENTS OF EUKARYOTIC CHROMATIN

Eukaryotic chromatin consists mainly of DNA, histones and non-histone proteins (NHP) with small amounts of RNA and other components. DNA occurs as a double stranded helix with complementary bases paired and the base-pairs stacked in the centre of the helix. DNA can adopt a variety of structures with different angles of the base pairs to the helix axis and different pitches to the helix. DNA adopts the B-form in free solution with the bases at right angles to the helix axis and 10.4 base pairs per turn of the helix. It is possible that specific DNA sequences, especially when complexed with specific proteins, might form other structures. Histones are basic proteins which fall into five major classes, H1, H2A, H2B, H3 and H4. They are responsible for maintaining the basic repeating structure of chromatin. Histones H3 and H4 have highly conserved amino acid sequences. The sequences are highly assymmetric with very basic N-terminal regions and aromatic, apolar and acidic residues in the C-terminal regions. Histones H2A and H2B are also highly conserved although they vary more than H3 and H4. H2A and H2B have very basic terminal regions with the apolar residues clustered in the central sections. Histone H1 at about 220 residues is about twice as large as the other histones. Its sequence is also more variable although a central, hydrophobic, region is conserved. The terminal regions are highly basic, especially the C-terminal half of the molecule. Histones form a specific octameric complex containing 2 each of H2A, H2B, H3 and H4 which forms the protein core of the nucleosome. Histones can be modified chemically after synthesis by phosphorylation, acetylation, methylation and diphosphoribosylation at specific sites in the amino acid sequence. Phosphorylation and acetylation are involved in conformational transitions of chromatin. Non-histone proteins are also involved in conformational transitions in chromatin. The HMG non-histone proteins are being characterized and may be involved in the structure of transcriptionally active chromatin.

CHAPTER 2 CHROMATIN STRUCTURE

The basic sub-unit of chromatin structure is called the nucleosome. It contains nine histone molecules namely the octamer core plus one H1, with approximately 195 base pairs of continuous DNA. The DNA length varies between

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organisms, between cell types and within one cell type. Within the nucleosome the octamer core is closely associated with 145 base pairs of DNA to form the core particle, which does not vary. Non-specific nucleases have been used to isolate nucleosomes and core particles and to probe their structure. Deoxyribonuclease-1 makes single strand breaks ('nicks') preferentially at sites about 10.4 bases apart, implying the DNA is coiled on the outside of the nucleosome in a modified B form structure. Deoxyribonuclease-1 preferentially attacks DNA in chromatin that is being transcribed. Neutron scattering techniques can distinguish between DNA and protein in the core particle and have been used to show that the core particle is a flat disc about 6 nm thick and about 11 nm in diameter. The outer part of the disc is mostly DNA; the inner part protein. X-ray diffraction studies of crystals of core particles lead to a similar model and should provide further details in future. The main features of the core particle structure can be obtained with only H3 + H4 + DNA. Nucleosomes can coil into a filament of 33 nm diameter which is stabilized by Mg^{2+} ions and by histone H1. Further coiling or packing is thought to occur, maybe stimulated by phosphorylation of H1. The final stage of chromosome packing occurs in the metaphase chromosome where loops or domains of DNA are organized on a protein matrix or 'scaffold'. Some features of metaphase chromosomes can be revealed by specific staining or 'banding' patterns. The conformational transitions of chromatin probably involve histone acetylation and phosphorylation.

CHAPTER 3 THE GENOMES OF BACTERIA, VIRUSES, PLASMIDS, MITOCHONDRIA AND CHLOROPLASTS

In comparison with eukaryotes, prokaryotes and subcellular genetic factors have very small genomes which are consequently utilized in a very economic way. *E. coli* probably has fewer than 1800 genes and viruses anything from 3 to 150. Gene regulation in prokaryotes is largely by operon-like arrangements of genes, and DNA synthesis is often continuous in a growing culture, both features distinguishing these cells from eukaryotic counterparts.

The organization of prokaryotic DNA to form a protein/DNA chromatin complex is probable although still somewhat uncertain, but viruses resident in the nuclei of eukaryotic cells are clearly organized into nucleosome complexes by utilizing the normal eukaryotic histones.

Economies practised by bacteria and viruses in utilizing their genomes are interesting and numerous, including regular use of both strands of a double stranded DNA molecule, common leader sequences for some 'mosaic' viral messenger RNAs, and overlapping transcription of the same DNA to yield different proteins by shifting the reading frame. The genomes of some viruses

have now been entirely sequenced, as have quite large tracts of the *E. coli* genome.

CHAPTER 4 SEQUENCE AND GENE ARRANGEMENTS IN DNA

The DNA content of a haploid cell (the C-value) varies widely between bacteria and higher eukaryotes. In bacteria it corresponds roughly to the amount of DNA required to code for proteins and immediate control sequences but in higher eukaryotes the C-value is much larger than required to code directly for the 5000 to 50 000 genes expected in a higher eukaryote. DNA reassociation experiments show that bacterial DNA has largely single copy DNA sequences but higher eukaryotes have substantial amounts of reiterated DNA sequences, which fall into three classes: middle repetitive DNA; highly repetitive DNA and inverted repeat DNA. Some of these sequences are clustered and can be isolated as satellites in equilibrium density gradients. Some highly repetitive DNA's have been sequenced and have a short basic repeat of 4 or more bases which is repeated with minor variations. They can often be located in metaphase chromosomes by in-situ hybridization and many such sequences occur at the centromeres or at the ends of the chromosomes. In most higher eukaryotes the middle repetitive DNA is interspersed in short stretches with the single copy DNA although *Drosophila* has a different interspersion pattern with long stretches of uninterrupted single copy DNA. Some middle repetitive DNA is repeated genes but the function of the remainder is unknown. They may be involved in control processes. Nucleic acid sequences can be determined directly by using restriction nucleases to prepare specific fragments and end labelling with base specific degradation or interrupted synthesis to sequence the fragments. Sequences of up to several thousand base pairs have been determined in this way. Study of specific DNA sequences has been greatly facilitated by the ability to clone a specific sequence in a virus or plasmid vector and so prepare relatively large amounts of highly purified material. Detailed study of nucleic acid sequences has revealed some unexpected features, particularly overlapping genes in viruses, interrupted coding sequences in eukaryotes ('split genes') and rearrangement of DNA sequences during development.

CHAPTER 5 THE CELL CYCLE AND REPLICATION

During normal cell proliferation the chromosomes undergo mitosis which involves chromosome condensation, separation and de-condensation. The rest

of the cell proliferation cycle is divided into G1, S and G2 phases. Most histone and DNA synthesis occurs in S phase but there is probably a sequence of structural transitions in chromatin throughout the whole cycle. The normal diploid cell, in eukaryotes, can be converted into haploid cells, for reproduction, by meiosis. Crossing over and genetic recombination occur during mitosis. DNA synthesis is carried out by a semi-conservative procedure in which each new strand is 'copied' from one complementary pre-existing strand to give a double strand containing one old and one new single strand. Enzymatically, the process is complex with the DNA being synthesized in short segments which are then joined. The structural changes in chromosomes that occur during the cell cycle and during differentiation are correlated with post-synthetic modifications of histones, particularly phosphorylation of H1 and acetylation of H3 and H4. Phosphorylation of H1 at specific sites probably controls the packing of coils of nucleosomes, particularly during the initial stages of mitosis. Acetylation of H3 and H4 probably affects nucleosome interactions to allow transcription to occur. Transcriptionally active chromatin displays a nuclease resistant DNA repeat similar to that in bulk chromatin. However, the nucleosome structure of transcriptionally active chromatin is different, particularly in its high sensitivity to DNase-I and rapid degradation to monomer nucleosomes by micrococcal nuclease.

CHAPTER 6 TRANSCRIPTIONALLY ACTIVE CHROMOSOMES

Both the lampbrush chromosomes of vertebrate oocytes and the giant polytene chromosomes of some Dipteran larval tissues are transcriptionally active, and have therefore made an outstanding contribution to our knowledge of gene function. Study of these structures has revealed that, in the first case, the active structural genes are located on the loops of DNA which are attached to the chromosome axis, and in the second, that they are found within the banded regions of the chromosomes. The correlation between genes and the loops or bands of these chromosomes is strong, but not absolute. The fact that these structures number some 2000 to 8000 per genome suggests that the true number of structural genes in these organisms may be close to these approximates. At a functional level, the two chromosome types suggest different models, since in the lampbrush chromosome most or all of the loops are active transcriptionally, but in the polytene chromosome puffing is restricted to a few bands at any one time. It is probable that the latter represents the more typical situation.

In the next chapter these models of chromosome function are discussed along with other information in a general consideration of gene regulatory mechanisms.

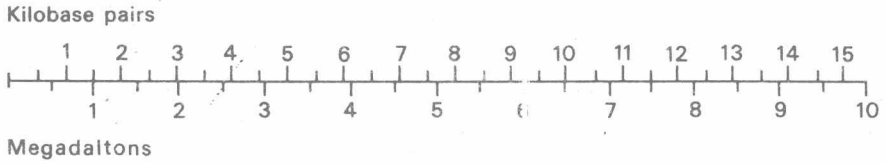
CHAPTER 7 CHROMATIN ACTIVITY AND GENE REGULATION

In this chapter we attempt to draw together the scattered and obviously partial evidence about gene regulation, together with the main theoretical contributions to this topic. While the operon model serves to illustrate effectively the normal mechanisms of gene regulation in prokaryotes, it is now obvious that it is not generally applicable to the eukaryotic situation. In higher cells quite a lot of the emphasis in gene control is at a post-transcriptional level i.e. processing of RNA. The transcriptional level of control is presumed to be mediated by special gene regulatory molecules, RNA or protein, which coordinate and specify the precise aspects of gene expression which constitute the basic mechanisms underlying eukaryotic cell differentiation.

Abbreviations

ATP	adenosine triphosphate
Å	angstrom unit
bp	base pair
cDNA	complementary DNA
Hn RNA	heterogeneous nuclear RNA
HMG	high mobility group
kb (1000 bases length of DNA)	kilobases
Mdal	megadaltons (1 megadalton = 10^6 daltons)
mRNA	messenger RNA
mol wt	molecular weight
10^{-9} gram (ng)	nanogram
10^{-9} metre (nm)	nanometre
10^{-12} gram (pg)	picogram
pfu	plaque-forming unit

Scale for conversion between kilobase pairs or duplex DNA and molecular weight.



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Chapter 1
Components of Eukaryotic Chromatin

1.1 INTRODUCTION

So far most of our detailed understanding of how genes function has come from studies of prokaryotes. These are free-living unicellular organisms (bacteria and blue-green algae), in which the genetic material is distributed throughout the cell organism. Both bacteria and viruses are well-suited for laboratory investigation and the powerful combination of biochemistry and genetics has led to most of our current concepts concerning the primary structure, control and expression of their genes. Prokaryotes are the most simple forms of living organisms. Higher life forms, called collectively, eukaryotes, can be distinguished from prokaryotes by several features, in particular by the presence of a cell nucleus which separates their chromosomes from the cytoplasm. Also whereas we can roughly equate the DNA content of prokaryotes with the number of genes they are known to contain this is not the case with eukaryotes. Although there is a very crude correlation between DNA contents and the genetic complexities of eukaryotes it is a general observation that eukaryotes contain appreciably more DNA than can be accounted for by the number of genes thought necessary to specify the organism. The functions of the very large proportions of non-coding DNA are not understood at present, though as will be seen later there have been unexpected and very exciting discoveries concerning the complexity of eukaryotic DNA. A third major difference between prokaryotes and eukaryotes is the presence in the latter of a group of basic proteins called the histones which are strongly complexed with DNA in chromosomes.

There is an arbitrary division of eukaryotes into lower and higher eukaryotes. Nucleated single cell organisms such as yeasts, algae, and protozoa, are regarded as lower eukaryotes, as are simple forms of multicellular organisms, for example moulds and other simple fungi. Higher eukaryotes usually refer to what are recognizable as animals and plants. Between these two extremes there is a multitude of organisms and it would not be useful to classify them other than as eukaryotes.

Probably one of the most important series of unsolved problems in biology is concerned with the organization and function of eukaryotic chromosomes. There are closely interrelated structure–function problems: the sequence organization of eukaryotic DNA; the structure of inactive chromatin; the structural transition which occurs when genes become active and are transcribed; the determination, maintenance and control of active genes in differentiated tissue; the control of chromosome structure through the cell cycle; the dis-

assembly and reassembly of chromatin during DNA replication; the structure of metaphase chromosomes, etc.

Recently there have been major advances in this area of biological research and the solutions to several of these problems are now in sight. This fortunate situation is a consequence of major advances in biochemical techniques and preparative strategies.

1.2 COMPONENTS OF EUKARYOTIC CHROMATIN

Historically chromatin was the term used to describe the contents of interphase nuclei as visualized in the light microscope. More recently chromatin has been adopted by biochemists as a label for the deoxyribonucleoprotein complex isolated from cells and tissues.

The composition of eukaryotic chromatin is illustrated in Fig. 1.1. There are four major components: DNA; a group of five classes of basic proteins, histones, which are now regarded as major structural proteins of eukaryotic chromosomes; a large number of other chromosomal proteins so far poorly characterized and called non-histone proteins (NHP); and RNA. Some general rules have emerged from studies of the compositions of the nuclei of different cells. Firstly, the ratio of the amount of histone to that of DNA is relatively constant for all tissues and lies in the range from 1.1 to 1.3. This constancy and the close association of histones with DNA has led to many proposals concerning their role in all aspects of chromosome structure and function. Secondly, although the amounts of NHP are very variable with NHP:DNA ratios in the range 0.2–0.8 there appears to be a rough correlation of these amounts of NHP

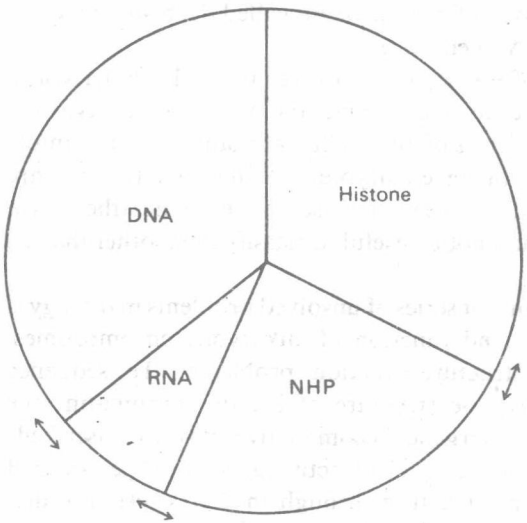

Fig. 1.1 Components of the eukaryotic chromatin. The histone to DNA ratio is kept constant at about 1.1 to 1.3. The arrows indicate variability in the amounts of RNA and NHP depending on the metabolic activity of the cell.

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