COLOR ATLAS OF CLINICAL HEMATOLOGY

COLOR ATLAS OF CLINICAL HEMATOLOGY

Molecular and Cellular Basis of Disease

FIFTH EDITION

A. Victor Hoffbrand
Emeritus Professor of Haematology, University College London
London, UK

Paresh Vyas
Professor of Haematology
Honorary Consultant Haematologist and Group Leader, MRC Molecular Haematology Unit, University of Oxford
Oxford, UK

Elías Campo
Senior Consultant, Haematopathology Unit, Hospital Clinic of Barcelona;
Professor of Anatomical Pathology, University of Barcelona
Barcelona, Spain

Torsten Haferlach
MLL Münchner Leukämielabor GmbH
Munich, Germany

Keith Gomez
Consultant Haematologist
Royal Free Hospital NHS Foundation Trust
London, UK

WILEY Blackwell

This edition first published 2019

Edition History
Churchill-Livingstone (1987); Mosby-Wolfe (1994); Mosby (2000); Mosby Elsevier (2009)

Registered Offices
John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA
John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial Office
9600 Garsington Road, Oxford, OX4 2DQ, UK

For details of our global editorial offices, customer services, and more information about Wiley products visit us at www.wiley.com.

Wiley also publishes its books in a variety of electronic formats and by print-on-demand. Some content that appears in standard print versions of this book may not be available in other formats.

A catalogue record for this book is available from the Library of Congress and the British Library.

9781119057017

Cover images: Courtesy of Victor Hoffbrand, Thomas Wagner, Torsten Haferlach, and Keith Gomez;
background image: Courtesy of Elías Campo
Cover design by Wiley

Set in 10/11pt Adobe Garamond by SPi Global, Pondicherry, India

10 9 8 7 6 5 4 3 2 1

CONTENTS

PREFACE

In the 9 years since the fourth edition of this *Color Atlas of Clinical Hematology* was published, tremendous advances have been made in the understanding of the pathogenesis of blood diseases. This is mainly due to the application of new molecular genetic techniques, including next-generation sequencing, to reveal the variants of DNA that underlie many of these inherited and acquired diseases. The World Health Organization (WHO) has incorporated this new knowledge in the 2016 Revised Classification of the Lymphoid and Myeloid Neoplasms. This Classification has been adopted in Chapters 13–24 of the present fifth edition of the *Atlas*. These cover the clinical and microscopic appearances and the immunologic, cytogenetic, and molecular genetic abnormalities that underlie these diseases. The references to the WHO 2016 Classification are:

- Swerdlow SH, Campo E, Pileri S, et al. The revision of the World Health Organisation classification of lymphoid neoplasms. *Blood* 2016;127:2375–2390.
- Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* 2016;127:2391–2405.
- Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, revised 4th edn. International Agency for Research on Cancer, Lyon, 2017.

The first three chapters of the new edition of the *Atlas*, written as previously by Paresh Vyas, are aimed at providing the reader with an understanding of normal cell machinery and of the molecular basis for such processes as DNA and cell replication, RNA species, trafficking and splicing, protein synthesis, transcription factors, growth factor signal transduction, epigenetics, cell differentiation, autophagy, and apoptosis. The subsequent 29 chapters describe and illustrate how these processes are disturbed in the various diseases of the bone marrow, blood, and lymphoid systems. Treatment is not usually described, except in Chapter 29 on thrombosis, but the *Atlas* includes diagrams which show the various points in the biochemical pathways where many of the new targeted therapies act.

Four new authors, all internationally renowned hematologists and pathologists, have updated the various sections of the book. Elías Campo (Barcelona), one of the lead authors of the WHO (2016) Classification, has rewritten the six chapters dealing with the lymphoid neoplasms. Torsten Haferlach (Munich) has added a new section explaining and illustrating the molecular techniques used for the diagnosis of the hematologic neoplasms. He has also collaborated substantially in rewriting the six chapters describing and illustrating the myeloid neoplasms. Keith Gomez (UK) has updated the text and added new illustrations and tables for the four chapters dealing with normal platelets, normal blood coagulation, and the bleeding and thrombotic disorders. Stefano Pileri (Bologna), also a lead author of the WHO 2016 Classification, has updated the chapter on histiocytic diseases. I am grateful to all four of these distinguished colleagues for taking on the task of bringing the chapters so expertly up to date despite their other heavy commitments.

While welcoming these new authors, I wish to express an enormous professional and personal tribute to John Pettit, who co-authored all four of the previous editions of the *Atlas*. John joined me at the Royal Free Hospital in 1975 when the Department of Haematology was in its infancy. We were confronted for the first time in our careers with teaching undergraduate medical students. We collaborated in writing handouts for the students as teaching aids. These were expanded to become a new undergraduate textbook, *Essential Haematology*, first published in 1980 by Blackwell Scientific.

In 1976 John and I set about collecting photographs of interesting clinical and microscopic appearances of blood diseases and assembled these as a new atlas, *Clinical Haematology Illustrated*. This was published in 1987 by Gower Medical Publishing. John returned from London to his native New Zealand in 1977 but we subsequently collaborated by mail and fax. John spent at least a week every year in London so we could work together on new editions of both books.

John was a great teacher as well as first class laboratory and clinical hematologist. His clear style of writing in succinct sentences, combined with his beautiful simple line diagrams and well-chosen clinical and microscope images of the highest quality, has contributed substantially to the success of both books. John retired from authorship of *Essential Haematology* after the fifth edition and of this *Atlas* after the fourth 2009 edition, but his major influence on style and content of both books, as well as the superb images he acquired for them, remain.

John Pettit

We are grateful to our new publishers Wiley Blackwell for their unstinting support and especially to Claire Bonnett and Magenta Styles in encouraging and supporting this pan-European team of authors and to Jennifer Seward in expertly editing all our manuscripts and assembling the book, including artwork, to a standard in which it could be published. We hope the book in its printed and electronic forms can be used as previously as an illustrated encyclopedia of normal blood and bone marrow processes and of blood diseases.

Victor Hoffbrand
London, 2018

CHAPTER

MOLECULAR BIOLOGY OF THE CELL

1

The aim of the first chapter is to provide a primer covering our understanding of the basic molecular and cellular processes of the cell, which inform a scientific understanding of hematologic diseases.

COMPARTMENTALIZATION OF THE CELL

A central evolutionary advance was the compartmentalization of cells, as shown in Fig. 1.1. The cell is bounded by a complex cell membrane that allows regulation of molecules into and out of the cell. Within the cytoplasm a number of different organelles perform key functions. For example, as described later in this chapter, mitochondria are critical for adenosine triphosphate (ATP) generation and heme biosynthesis. Proteins are translated from amino acids and undergo post-translational modification in the Golgi complex and rough endoplasmic reticulum. Depending on the cell type, there are specialized structures within the cytoplasm that allow the cell to perform its specialized role.

THE NUCLEUS

As we focus in on the nucleus, it is clear that it is also bounded by a specialized nuclear envelope and membrane (Fig. 1.2). Entry and exit out of the nucleus is regulated by nuclear pores. Within the nucleus, deoxyribonucleic acid (DNA) is tightly packaged by proteins and the DNA/protein complex is known as chromatin. Chromatin has different appearances under light or electron microscopes. When DNA is tightly packaged (and the genes more likely to be not expressed), it is known as heterochromatin. Under the light/electron microscope it appears darker. When DNA is less tightly packaged it is called euchromatin and is lighter in appearance. The other visible structure within the nucleus, in some cells, is the nucleolus, where ribosomal genes are transcribed and assembly of the ribosome takes place (as discussed later).

The DNA in the nucleus is distributed among 22 pairs of autosomal chromosomes (numbered 1–22, in order of size) and two sex chromosomes (Fig. 1.3A). When cells are in the metaphase phase of the cell cycle, chromosomes condense and can be visualized by a technique called karyotyping. Chromosomes are divided into two arms: a short arm, termed

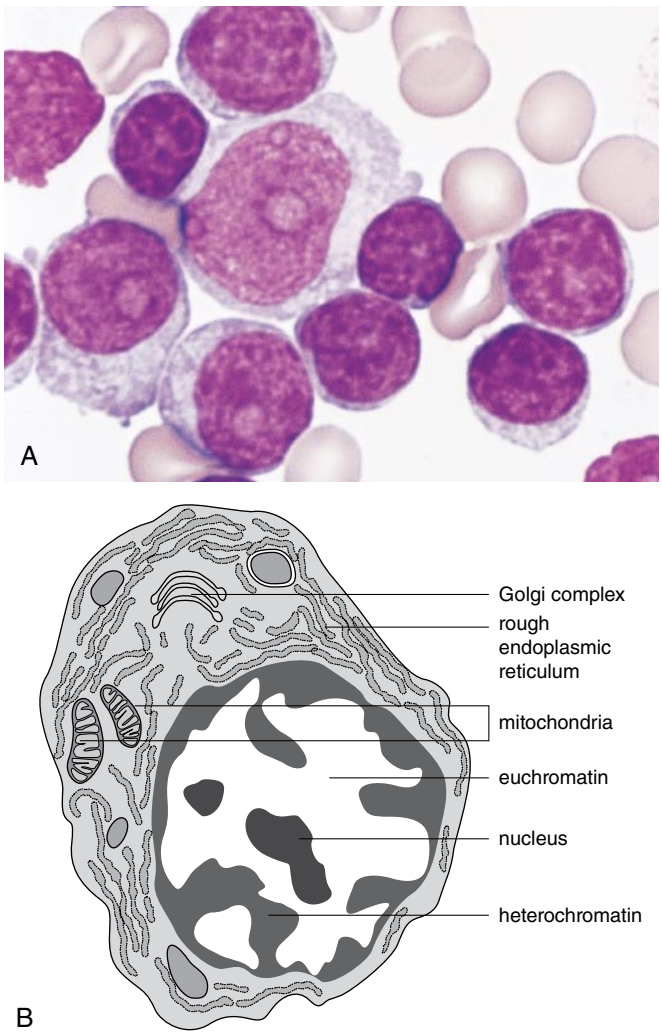

Fig. 1.1. A, Photomicrograph showing the morphology of many cells with prominent nucleoli, in this case, B cells. **B,** A schematic representation of the intracellular composition as visualized by electron microscopy. The nucleus is composed of euchromatin, which is less condensed, paler, and more transcriptionally active, and heterochromatin, which is more condensed, darker, and less transcriptionally active. In cytoplasm subcellular organelles including mitochondria, rough endoplasmic reticulum, and the Golgi complex are shown. The function of these organelles is discussed later. (Courtesy of Professor JV Melo.)

Color Atlas of Clinical Hematology: Molecular and Cellular Basis of Disease, Fifth Edition. A. Victor Hoffbrand, Paresh Vyas, Elías Campo, Torsten Haferlach, and Keith Gomez

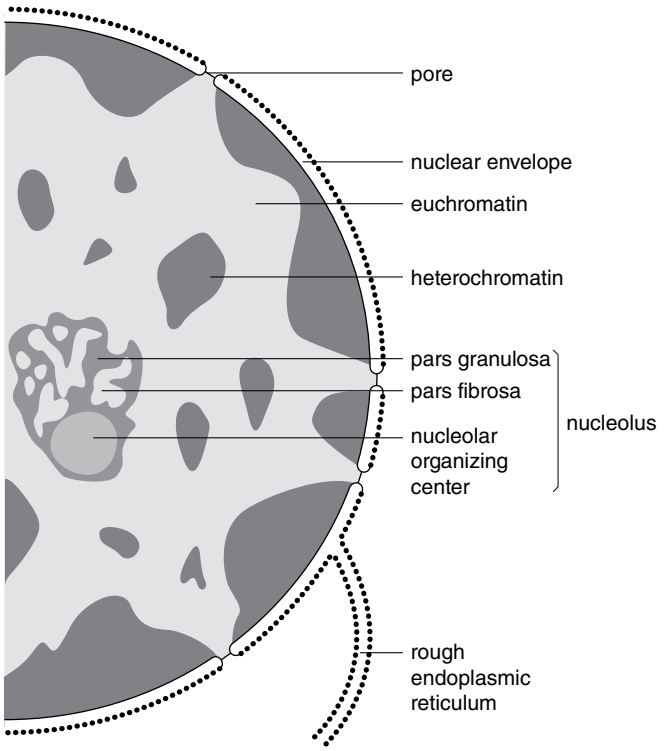

Fig. 1.2. Schematic representation of a portion of the nucleus. The nucleus is highly compartmentalized, containing specialized structures. The nucleolus is composed of a pars granulosa, a pars fibrosa, and a nucleolar organizing center and makes transfer RNA. The nucleus is bounded by a nuclear envelope that is lined by rough endoplasmic reticulum. There is controlled entry and exit into the nucleus via nuclear pores.

p, and a longer arm, q. The region where the chromosomes join is termed the centromere. Chromosomes are further subdivided into light and dark bands (depending on how they stain with the Giemsa dye) (Fig. 1.3B). When cells are not in metaphase, chromosomes are more diffusely spread through the nucleus. Most current evidence suggests that the chromosomes occupy discrete territories (chromosomal territories) within a nucleus (Fig. 1.3C). These territories need not be contiguous and can be shared with other chromosomes. However, there are still many aspects of how chromosomes are organized that remain unclear. For example, what constrains chromosomes to territories and how do territories affect gene regulation? Recent work suggests that within chromosomal territories chromatin exists in topologically associated domains (TADs) and that actively expressed genes along the chromosome and possibly even from different chromosomes may congregate in specialized structures where RNA is made from (transcribed) from genes. This process is called transcription and the specialized structures are known as transcription factories (see later).

The sequencing of the human genome was a landmark in biology. It allowed all the human genes arrayed along the chromosomes to be catalogued (Table 1.1). Genes are divided into protein-coding genes (of which there are ~21 000), genes that encode different types of RNA (e.g. ribosomal RNA, micro-RNAs, small nuclear RNA), and RNA moieties that are not translated into a functional protein or RNA (pseudogenes). The genome also dedicates sequence to other RNA species that do not make protein but that regulate either transcription or the production of protein from RNA (a process known as translation). These RNA sequences include micro-RNAs, long and

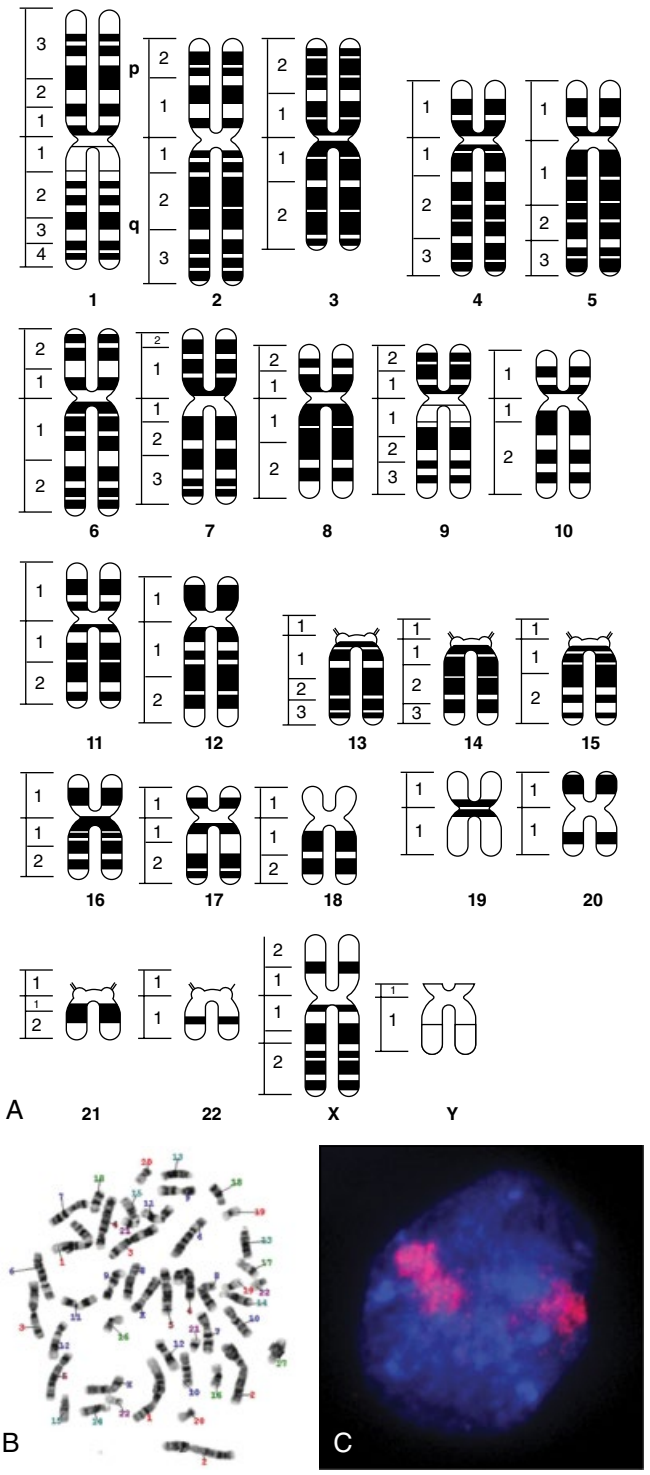

Fig. 1.3. A, DNA in the human nucleus is organized into 46 chromosomes. There are two copies of chromosomes 1–22 with two sex chromosomes (XX or XY). Each chromosome is divided into a short arm (p) and a long arm (q) and then subdivided into major numeric subsections. For example, the short arm of chromosome 1 (1p) has three subsections and the long arm (1q) has four subsections. **B,** The gross subdivision of chromosome can be visualized by Giemsa staining of chromosomes that have been subject to brief proteolytic cleavage. (B, Courtesy of Professor H Lodish.) **C,** Within an interphase nucleus chromosomes occupy discrete territories. The figure shows the territory occupied by chromosome 11 (red color) in a primary erythroblast. (C, Courtesy of Jo Green and Dr. Veronica Buckle.)

TABLE 1.1. ALL THE GENES AND OPEN READING FRAMES IN THE HUMAN GENOME HAVE BEEN CHARACTERIZED FROM THE SEQUENCING OF THE HUMAN GENOME. THIS TABLE SHOWS THE SIZE OF EACH CHROMOSOME (IN MEGABASES) AND NUMBER OF GENES AND PSEUDOGENES ON EACH CHROMOSOME.

Chromosome number	Size (Mb)	Gene	Pseudogene
1	248.96	5,078	1,372
2	242.19	3,862	1,166
3	198.3	2,971	887
4	190.22	2,441	799
5	181.54	2,578	766
6	170.81	3,000	876
7	159.35	2,774	896
8	145.14	2,152	661
9	138.4	2,262	702
10	133.8	2,174	631
11	135.09	2,920	835
12	133.28	2,521	680
13	114.36	1,381	477
14	107.04	2,055	583
15	101.99	1,814	555
16	90.34	1,920	451
17	83.26	2,432	541
18	80.37	988	295
19	58.62	2,481	514
20	64.44	1,349	329
21	46.71	756	202
22	50.82	1,172	348
X	156.04	2,158	859
Y	57.23	577	395
MT	0.016569	37	–

short noncoding RNAs. There are also sequences dedicated to regulating transcription of individual genes or banks of genes; these are called promoters and enhancers. This provides a primary description of our genetic makeup. The characterization of the human genome is still being refined as we understand more about how genes are organized and how transcriptional expression and protein translation is controlled.

Genes themselves are composed of DNA, which is made up of four nucleotides. Each nucleotide consists of a phosphate group linked by a phosphoester bond to a pentose sugar molecule (ribose) that lacks a hydroxyl group (thus it is deoxyribose), which is then attached to one of four heterocyclic carbon- and nitrogen-containing organic rings: adenine (A), cytosine (C), guanine (G) and thymidine (T). C and T are known as pyrimidines and A and G as purines. These are then linked together into polynucleotides via phosphoester bonds. As James Watson and Francis Crick correctly proposed, these are organized into two associated antiparallel polynucleotide strands that have a 5′ to 3′ direction and form a double helix. The strands are held in register by base-pairing between the two strands such that each A is paired with a T via two hydrogen bonds and each C with a G via three hydrogen bonds. Hydrophobic and van der Waals interactions combine with the thousands of hydrogen bonds to give the double helix great stability. In the common "B" form, the helix is right handed and makes a complete turn every 3.4 nm (about 10 base pairs) (Fig. 1.4A,B). The space between the strands creates a major and minor groove. In low humidity, DNA can adopt a more compact form with 11 base pairs per helical turn ("A" form) (Fig. 1.4C). Finally, short stretches of DNA composed of alternate purines and pyrimidines can form an alternate stacked Z structure.

GENE TRANSCRIPTION AND MESSENGER RNA TRANSLATION: THE PRODUCTION AND JOURNEY OF mRNA

A copy of the DNA of genes is transcribed into RNA by transcription in the nucleus. RNA is processed and transported into the cytoplasm. RNA corresponding to protein genes is then translated in the cytoplasm. Not surprisingly, these processes are very complex, affording opportunities for the cell to exquisitely regulate the complement of proteins made but also vulnerable to errors that lead to disease.

Genes are transcribed by one of three different RNA polymerases (RNA Pol I, II, and III). RNA Pol II transcribes most protein-coding genes. The remaining genes are transcribed by RNA Pol I and III. These include genes encoding ribosomal RNAs (makes ribosomes, see later), small nuclear RNAs (involved in processing RNA in a process called splicing, see later), and some transfer RNAs (involved in protein translation, see later). Pol I- and Pol II-transcribed genes will not be discussed in detail further in this book. However, it is important to remember that in a typical rapidly growing mammalian cell, ~80% of total RNA is ribosomal RNA and ~15% is transfer RNA.

When RNA is transcribed, a gene is said to be "expressed." Transcription of each gene begins at the 5′ end of the gene at its transcriptional start site (TSS) (Fig. 1.5). For any one gene the TSSs can either be single or multiple over several neighboring nucleotides. The DNA sequence 5′ of the gene helps to regulate transcription and is known as the promoter. This sequence works with other sequences (called regulatory sequences or *cis*-elements, see later) to provide finely tuned control over the amount of mRNA produced. In Chapter 9 the regulatory sequences involved in globin gene expression are described.

The body of the gene is segmented into exons separated by intervening sequences (introns). The exonic sequence is divided into protein-coding and noncoding sequences. RNA Pol II makes a RNA copy of the whole of the gene (primary transcript). This RNA species is then processed within the nucleus. As the nascent elongating primary transcript is produced a 5′ 7-methylguanine cap is added to the 5′ end to protect the RNA from enzymatic degradation. In addition, as nascent RNA transcript (heterogeneous RNA [hnRNA]) emerges from the RNA Pol II, it is sheathed in a large set of nuclear proteins in structures called heterogeneous ribonuclear particles (hnRNPs). hnRNP-associated proteins are important for transport of the RNA species and probably aid in the processing of RNA. Once the primary transcript is made, the 3′ end of the transcript is recognized by a protein complex that includes an enzyme called an endonuclease that cleaves the RNA transcript to produce a 3′

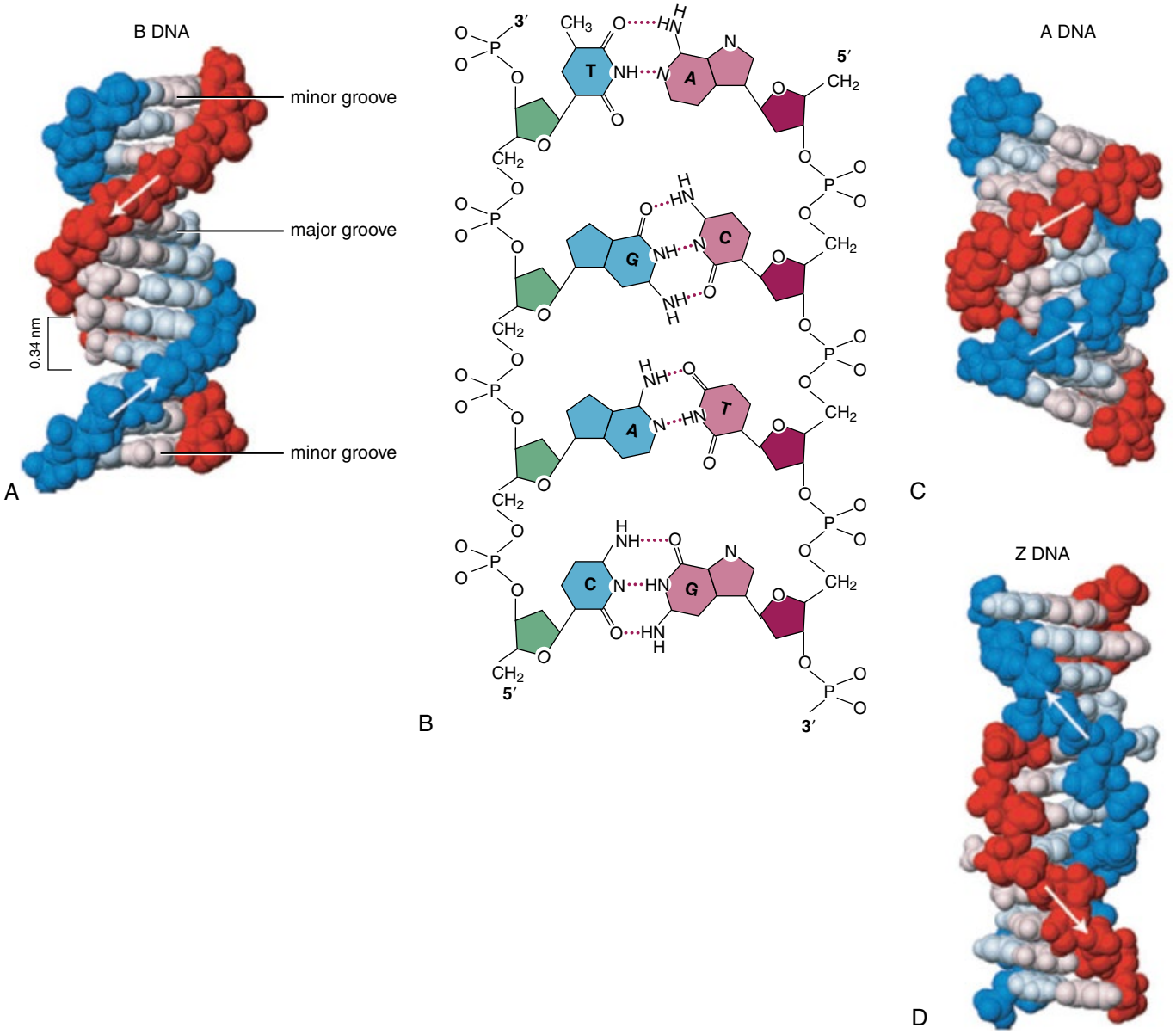

Fig. 1.4. Models of various structures adopted by DNA. **A,** Space-filling model of the "B" form of DNA. This is the common form of DNA, with a helical turn every 10 base pairs. The major and minor grooves are visible. **B,** Stick model shows that DNA is composed of a sugar phosphate backbone with the bases ("A," "C," "G," and "T") pointing inward (blue and light brown). **C,** More compact "A" form of DNA with 11 bases pairs per helical turn. **D,** "Z" form of DNA is a left-handed helix. (Courtesy of Professor H Lodish.)

end of the RNA. This then allows the enzyme polyadenylate polymerase to attach a homo-polymeric string of A residues to the 3′ end of the transcript called the poly-A tail. Increasing recent evidence suggests that the processes of transcription initiation, elongation by RNA Pol II, and 3′ end processing may be co-regulated.

The introns are then spliced out to form the mature mRNA species (a simplified version of this process is presented in Fig. 1.6). Splicing is an elaborate process that involves a large number of steps catalyzed by a splicing complex (or spliceosome), which contains small nuclear RNAs (snRNAs) and proteins, and that produces small nuclear ribonuclear particles (snRNPs). It is estimated that over 100 proteins are involved in splicing. At first approximation, this process is probably as complex as regulation of transcriptional initiation and translation. One reason why a cell invests this degree of effort into splicing is that it allows a cell to generate multiple different mRNA species from a single gene, contributing to the biological complexity that an organism can achieve from a limited gene set. However, the genes encoding proteins controlling splicing can acquire mutations and the mutant proteins may cause aberrant splicing, leading to hematologic diseases. One important part of splicing is that the two nucleotides that lie in the intron and mark the boundary of an exon–intron are almost always invariant (Fig. 1.6). Thus, the 5′ end of the intron is usually marked by the dinucleotide "GU" whereas the 3′ end has "AG."

Like hnRNA, mRNA is wrapped in chaperone proteins to form mRNPs (messenger ribonuclear particles) that are exported from the nucleus through a water-impermeable phospholipid bilayer, the nuclear envelope that is studded with proteins and pores (Figs. 1.7 and 1.8). The nuclear pore complex (NPC) is a large structure (~125 million Da), about 30 times the size of the ribosome. It is made of multiple copies of a large number (~100)