The ACT Cytogenetics Laboratory Manual

Second Edition

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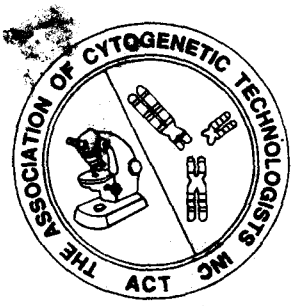

Editor

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The Association of Cytogenetic Technologists
Houston, Texas

RAVEN PRESS NEW YORK

Raven Press, Ltd., 1185 Avenue of the Americas, New York, New York 10036

Made in the United States of America

Library of Congress Cataloging-in-Publication Data

The ACT cytogenetics laboratory manual. — 2nd ed. / editor, Margaret J. Barch.
p. cm.
"Also known as the ACT technical manual"—Pref.
Rev. ed. of: Cytogenetics laboratory manual / edited by Marilyn Schaeffer Hack and Helen J. Lawce. [c1980].
Includes bibliographical references and index.
ISBN 0-88167-774-4
1. Human cytogenetics—Laboratory manuals. I. Barch, Margaret J. II. Association of Cytogenetic Technologists. III. Cytogenetics laboratory manual. IV. Title: ACT technical manual.
[DNLM: 1. Cytogenetics—laboratory manuals. QH 583.2 A188]
RB44.A38 1991
616'.042—dc20
DNLM/DLC
for Library of Congress
91-15516.
CIP

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Preface

The *ACT Cytogenetics Laboratory Manual* (also known as the *ACT Technical Manual*) was originally published by the Association of Cytogenetic Technologists, Inc., in 1980. Two additional chapters were published separately in 1984 and 1985. A prodigious cooperative effort, the first edition is used and loved for its wealth of basic information, techniques contributed from laboratories all over the country, and advice on the use of those techniques.

I have sought to preserve in the second edition the good qualities of the first. However, I have also added a needed index, updated and expanded chapters, and added new chapters on current areas of interest and concern to cytogeneticists. Chapter One, The Cell and Cell Division, has been enlarged to provide a brief introduction to molecular techniques and may thus be helpful to established technologists as well as to students. Instead of one tissue culture chapter, this edition has separate chapters on peripheral blood culture, continuous cell lines, and prenatal diagnosis and culture. New chapters are presented on solid tumors, fragile sites, and molecular cytogenetics. The harvesting and slide-making chapter now encompasses chromosome elongation techniques. Revised chapters on microscopy and photography and analysis are included, as are new chapters on chromosome banding and safety and quality control.

Some protocols are grouped together at the end of the relevant chapter; some are integrated with the text if the author thought the placement was more logical. Several chapters give one illustrative protocol within the text and supplementary ones at the end. In each instance we have tried to offer a variety of techniques to provide a choice for experienced technologists but, at the same time, to not overwhelm the novice. The International System of Units* has been used throughout, and we have made an effort to ensure that protocols conform to NCCLS guidelines.†

I have endeavored to produce a second edition that would be a worthy successor to the groundbreaking original and to pave the way for the efficient publication of future updates. If I have succeeded, this edition will contribute to the future as well as to the present.

Margaret Johnson Barch

* Lundberg GD, Iverson C, Radulescu G. Now read this: the SI units are here. *JAMA* 1986;255(17):2329–2339.

† Bender JL, Bettner B, Blank CH, Hassemer D, Moran JJ, Stevens JE. *Clinical laboratory procedure manuals 4(2)*. Pennsylvania, National Committee for Clinical Laboratory Standards, 1984.

Acknowledgments

I would like to thank all who contributed to this work—the authors, those who provided protocols, those who answered the prepublication survey, Jack Spurbeck and Helen Lawce who provided color photographs for the frontispiece, and Vivegen, Inc. who sponsored color photographs in the Molecular Cytogenetics chapter. I also thank the ACT directors past and present who helped and encouraged me, especially Turid Knutsen, Barbara Kaplan, and Kay Dale; the many members who offered words of appreciation and support, especially to Helen Lawce who always was able to inspire and encourage me; Kimberly Hayes, who lent a hand early on; and Catsie Tucker and the Manual Task Force, who aided in the selection of a publisher. A special thank you to Gary Barch, whose love sustained me throughout this project and whose helpful ideas and efforts got many a job done.

Contents

The ACT Cytogenetics Laboratory Manual, Second Edition, edited by M. J. Barch. The Association of Cytogenetic Technologists © 1991.
Published by Raven Press, Ltd., New York.

CHAPTER 1

The Cell and Cell Division

Helen J. Lawce and Margaret J. Barch

THE CELL

The cell is the basic unit of life—the simplest structure capable of independent existence. The cell, in general, consists of cytoplasm bound by a cell membrane and of a centrally placed nucleus, also enclosed in a membrane. Higher organisms are composed of complex colonies of interdependent cells, each colony with a specialized function necessary for the survival of the organism. Cells that have the same general function are often grouped together to form tissues, such as muscle, bone, and connective tissue. Tissues may be combined in larger functional units called organs, such as kidneys, skin, and heart. Organs can in turn be grouped by function into organ systems, such as the respiratory and circulatory systems.

Chemical Content of Cells

Cells consist of water, inorganic ions or molecules, and a variety of organic compounds. Among the inorganic molecules are potassium, sodium, magnesium, and calcium. Trace amounts of many heavy metals are also present, as are bicarbonate and phosphate. In many ways, cytoplasm resembles a colloid, with particles suspended in a continuous gel-like phase. The large organic molecules (called macromolecules), which give the cytoplasm these colloidal properties, can be grouped into three main classes: proteins, nucleic acids, and polysaccharides. Each class is a polymer built with a different subunit (monomer): Proteins are made up of amino acid subunits, nucleic acids are polymers of nucleotides, and polysaccharides are composed of sugar monomers.

Proteins carry out several important functions within the cell, including structural support, catalysis of metabolic reactions, and regulation of complex cellular processes. Examples of structural proteins are actin and myosin in muscle and keratin in hair, nails, and hooves. Regulatory proteins include hormones, growth factors, and receptors.

The nucleic acids are involved with protein synthesis and the storage of genetic information. There are two kinds of nucleic acids: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), each a polymer of nucleotides. Nucleotides consist of a purine or pyrimidine, a five-carbon sugar, and a phosphate group. The sugar in DNA is deoxyribose; in RNA it is ribose.

The polysaccharides function as food storage molecules and as structural molecules. The two most important polysaccharide food reserves in higher organisms are starch and glycogen, both of which are polymers of glucose sugar. Structural polysaccharides include cellulose and chitin: Cellulose is the major constituent of cell walls in plants, and chitin is found in the exoskeletons of insects and crustaceans.

Another important organic molecule, although it is not classified as a macromolecule, is the lipid. Lipids encompass a diverse group of compounds that are all soluble in nonpolar, organic solvents. Included in this class are fats, which are used primarily for energy storage; phospholipids, which are found in cell membranes; sphingolipids, which are especially prominent in the cell membranes of brain and nervous tissue; glycolipids, which are important in the myelin sheath of nervous tissue; steroids, including male and female sex hormones, bile acids, adrenocortical hormones, and cholesterol; and fatty acids, which are components of energy storage molecules.

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Structural Organization of Cells (1,14)

The cell membrane, or plasmalemma, defines the cell boundary (see Fig. 1). Cell membranes consist primarily of phospholipids and proteins. The phospholipids form a bimolecular layer, with their hydrophilic ends at the outer surfaces of the membrane and their hydrophobic chains extending into the middle of the membrane. The protein components of the membrane are globular particles distributed through the lipid bilayer; their polar amino acids may be exposed on an outer surface, but nonpolar portions remain in the interior. The cell membrane serves as a physical barrier for the cell contents. If one were to tear a hole in this membrane by micromanipulation, the contents would spill out into the surrounding medium. An intact cell can rapidly repair minor membrane damage, but more extensive damage leads to cell death. The membrane also acts as a regulatory barrier for the entry and exit of molecules and particles. This ability to regulate the passing of substances is called *selective permeability*.

Substances can cross the cell membrane by three mechanisms: by free diffusion along a gradient, meaning that substances travel from regions of high concentration to regions of lower concentration; by active transport, which requires energy and moves substances against a concentration gradient; and by enclosure in vesicles that move substances into the cell (endocytosis) or out of the cell (exocytosis). Water can move freely across cell membranes in both directions, and this property allows hypotonic solutions (those less concentrated than the inside of the cell) to swell

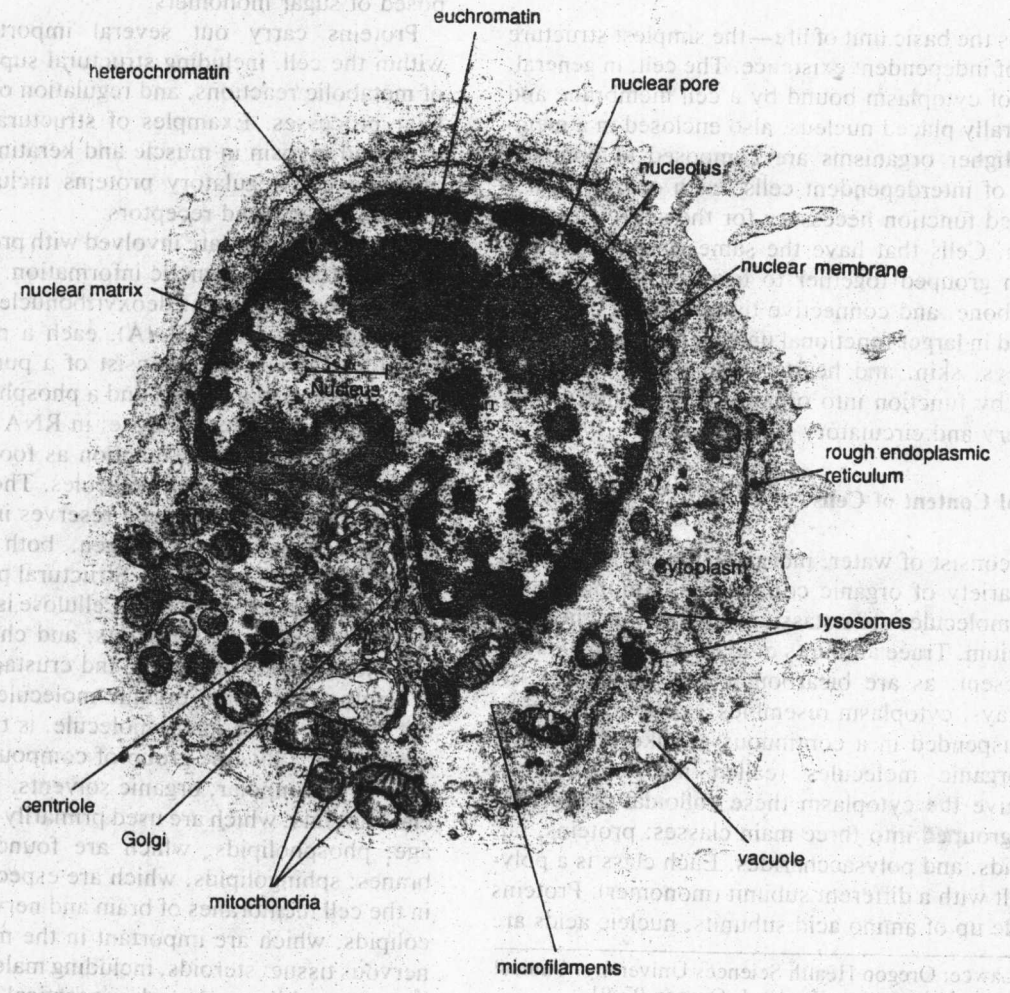

FIG. 1. The cell. (Electron micrograph by Garland Yee.)

the mitotic cell, facilitating chromosome spreading for cytogenetic study.

Molecules of glycoprotein (proteins with sugar molecules attached at points along the amino acid chain) exist on the surface of the protein-lipid membrane and sometimes project through it into the cell. These glycoproteins function in cell adhesion, both to other cells and to culture flask surfaces. Trypsin, a protease (an enzyme that digests proteins), removes these molecules, therefore freeing cells for subculture or harvest. Glycoproteins can also be antigenic (e.g., in red cells they determine blood type), and they serve as receptors for viruses, plant agglutinins (e.g., phytohemagglutinin), and hormones. Glycoproteins are further implicated in contact inhibition, a process in which normal cells stop dividing as cultures become confluent. Tumor cells often lose this property and tend to keep growing unchecked in a disorganized fashion when the growth surface is limited. Glycoproteins on the cell surface are also important in cell-cell recognition. If lymphocytes are stripped of their glycoproteins, they no longer accumulate in the lymph nodes.

The cytoplasm of the cell, though unremarkable by light microscopy, reveals complex structures when viewed with an electron microscope (EM) (see Fig. 1). Tiny granules seen with a light microscope are shown to be a series of vacuolar structures bound by lipoprotein membranes similar to the cell membrane, some of which also contain a complex system of internal membranes. These structures include the following: The *endoplasmic reticulum* (*ER*) consists of membranous channels (smooth ER) that may contain ribosomes (rough ER). The *Golgi apparatus* is a region of the smooth ER that functions as a processing and packaging region for substances to be secreted from the cell. *Lysosomes* are bodies that contain powerful digestive enzymes and can dispose of native or foreign garbage. *Mitochondria*, organelles in which oxidation of nutrients (oxidative phosphorylation) occurs, provide energy to synthesize adenosine triphosphate (ATP). ATP conserves the energy from the oxidative reaction that would have been lost as heat and makes it available to the cell for work. Thus, the mitochondria have been called the powerhouses of the cell. The mitochondria have a double membrane: an outer membrane plus an inner membrane infolded into numerous projections called cristae, where oxidation of nutrients takes place. The mitochondria command special interest because they contain their own DNA and ribosomes.

All the single-membrane organelles of the cell are probably interconnected and continuous at some time, including the lysosomes, microbodies, Golgi apparatus, and the nucleus.

In addition to these membranous structures, cells contain other important structures. The ribosomes are tiny spherical bodies on which the synthesis of proteins takes place. They are found either free in the cytoplasm or attached to mitochondria, ER, or the outer surface of the nuclear membrane, and they are made up of 50 to 80 different proteins and 3 to 4 different kinds of RNA molecules. Polypeptide chains are made on groups of ribosomes called polyribosomes, or polysomes. The polysome contains a variable number of ribosomes held together by a messenger RNA (mRNA) strand. The mRNA strand determines the sequence of amino acids in the synthesized protein. Initiation, elongation, and termination of the polypeptide are determined by signals residing in the messenger RNA. Antibiotics such as streptomycin, chloramphenicol, and puromycin block protein synthesis at one of these three stages.

Also found in the cytoplasm are centrioles (or basal bodies), which are small granules or short rods usually found near the nucleus. Electron microscopy shows that centrioles are tubular with a regularly arranged series of 27 fibers, or tubules, in sets of three. Centrioles usually occur in pairs that are perpendicular to each other (a pair is called a diplosome), and they are self-duplicating bodies. The centrioles are responsible for the formation of spindle fibers, which help separate chromosomes to their respective daughter cells in cell division, and for the formation of the cilia and flagella, the external hairlike projections that function in cell motility. Spindle fibers, cilia, flagella, and centrioles are all made up of microtubules. Like their close relatives, the microfilaments, microtubules are involved in cell movement, cytoplasmic streaming, cell cleavage, and membrane invaginations. Microtubule-initiated motion almost always requires ATP as an energy source. Colchicine inhibits cells from completing mitosis by binding to the monomer tubulin, thereby blocking its assembly into polymeric spindle fibers. Colchicine also indirectly disassembles already-formed spindle fibers. Without spindle fibers, chromosomes are unable to move away from the metaphase plate and complete cell division.

THE NUCLEUS (3,4,6,8,11,13,14,15,18)

Bacteria and blue green algae carry their genetic material in the cytoplasm of the cell and are called prokaryotes. Other, more complex organisms have their genetic information organized within a nucleus. These plant and animal organisms, including humans, are termed eukaryotes (*eu* = true; *karyon* = nucleus).

In higher organisms, every cell has a nucleus at some stage of its existence. Some cells have more than one nucleus, and some, such as red cells and platelets, lose their nuclei when they mature. Cells lacking nuclei are severely limited in their metabolic activities. The nucleus is the site of the genetic material, DNA, which

determines the specific morphological, biochemical, and metabolic characteristics of the cell. The nucleus is also the site of ribosome precursor assembly.

The nucleus contains a nuclear membrane, chromatin, and nucleoli (see Fig. 1). The term *nuclear matrix* refers to the fibrous material that remains if the chromatin and nucleoli are extracted. The appearance of the nucleus is markedly different in interphase (nondividing) and mitotic (dividing) cells. In interphase, the nucleus is a conspicuous spherical body in the cell interior. It was first noted by Brown in plant cells in 1831 (5). By light microscopy, the nucleus appears as an amorphous network, called chromatin, of variably condensed fibers not distinguishable as individual entities. Highly condensed chromatin stains darkly with nuclear stains and is known as heterochromatin; the more dispersed chromatin, which stains lightly or not at all, is called euchromatin. In cell division, the chromatin condenses into deeply staining threadlike or rodlike structures called chromosomes (*chromo* = stain; *soma* = body), which are present in specific numbers in each cell of a given species. This process of chromatin condensation to form chromosomes during division is necessary for the equal parceling of genetic information to daughter cells.

Under the electron microscope, the chromatin and chromosomes appear as fibrous structures because they are comprised of DNA molecules that are themselves filamentous. Fibers of DNA with associated proteins are about 30 nanometers (nm) in diameter, but protein-depleted strands are only about 10 nm in diameter. Chromatin fibers with diameters greater than 30 nm are occasionally observed and are believed to represent coiling or folding of these main fibers.

Chemistry of the Nucleus

The nucleus contains the nucleic acids DNA and RNA along with structural and regulatory proteins. The DNA is the genetic material, and the RNA is responsible for carrying out the instructions coded by the DNA. The primary functions of the nucleic acids are gene replication, the process of copying sequences of DNA (genes) for distribution to daughter cells, and gene transcription, the process of copying sequences of DNA into complementary strands of RNA. These transcript RNAs may then be translated into corresponding sequences of amino acids during the synthesis of polypeptides (proteins). As previously discussed, protein synthesis occurs on cytoplasmic ribosomes.

Structure of the Nucleus

The Nuclear Membrane, or Envelope

The nuclear envelope, as the membrane surrounding the nucleus is called, is a porous double membrane with ribosomes attached to the outside. The outer membrane is continuous with the ER at many sites. The nuclear envelope disappears during cell division. Inside the nucleus, there are two obvious structural elements, the nucleolus and the chromatin.

The Nucleolus

The nucleolus is a darkly staining body placed eccentrically in the nucleus. There may be one to four nucleoli in the normal nucleus. The size of the nucleoli varies with the cell type and the metabolic state of the cell; they are larger in rapidly dividing cells and in cells active in protein synthesis. The nucleolus is the site of ribosome precursor assembly, and all the ribosomes in the cytoplasm originate in the nucleolus. Each nucleolus is formed along the nucleolar-organizing region (NOR) of one or more specific chromosomes and is recognizable during cell division. The nucleolus is comprised primarily of RNA and protein but contains some DNA.

The Chromatin

Chromatin is a complex material, intricate in its organization. It is composed of DNA, proteins (mainly histones), RNA, and certain polysaccharides.

DNA

Studies with sister chromatid exchange, electron microscopy, and other techniques give evidence that a chromatid, one of a pair of metaphase chromosome strands, contains a single, uninterrupted, highly folded molecule of DNA. DNA itself is a double helix made up of two strands. Each strand is comprised of nucleotides, each consisting of a sugar molecule, a phosphate group, and one of four bases: adenine (A), guanine (G), thymine (T), or cytosine (C). The nucleotides are arranged side by side, with two bases forming one rung of a twisted ladder, and the phosphate and sugar form the outer structure (Fig. 2). The sugar in DNA, deoxyribose, has five carbon atoms, the third and fifth of which are bonded together by a phosphate (phosphodiester) linkage. Thus, a single strand of DNA is a polymer of deoxyribonucleotides held together by a 3′-5′ phosphate linkage between their sugars. This is called the sugar-phosphate backbone of the DNA molecule, and it lies on the outside of the DNA fiber; the bases extend inward from the backbone. The free 3′ and 5′ ends give the molecule a polarity, or direction. Watson and Crick (26b,c) determined the secondary structure of DNA, that two strands of these polynucleotides form a helix with the polarity of the strands in opposition and the bases on the inside. The bases hold the two strands together by hydrogen bonds (see

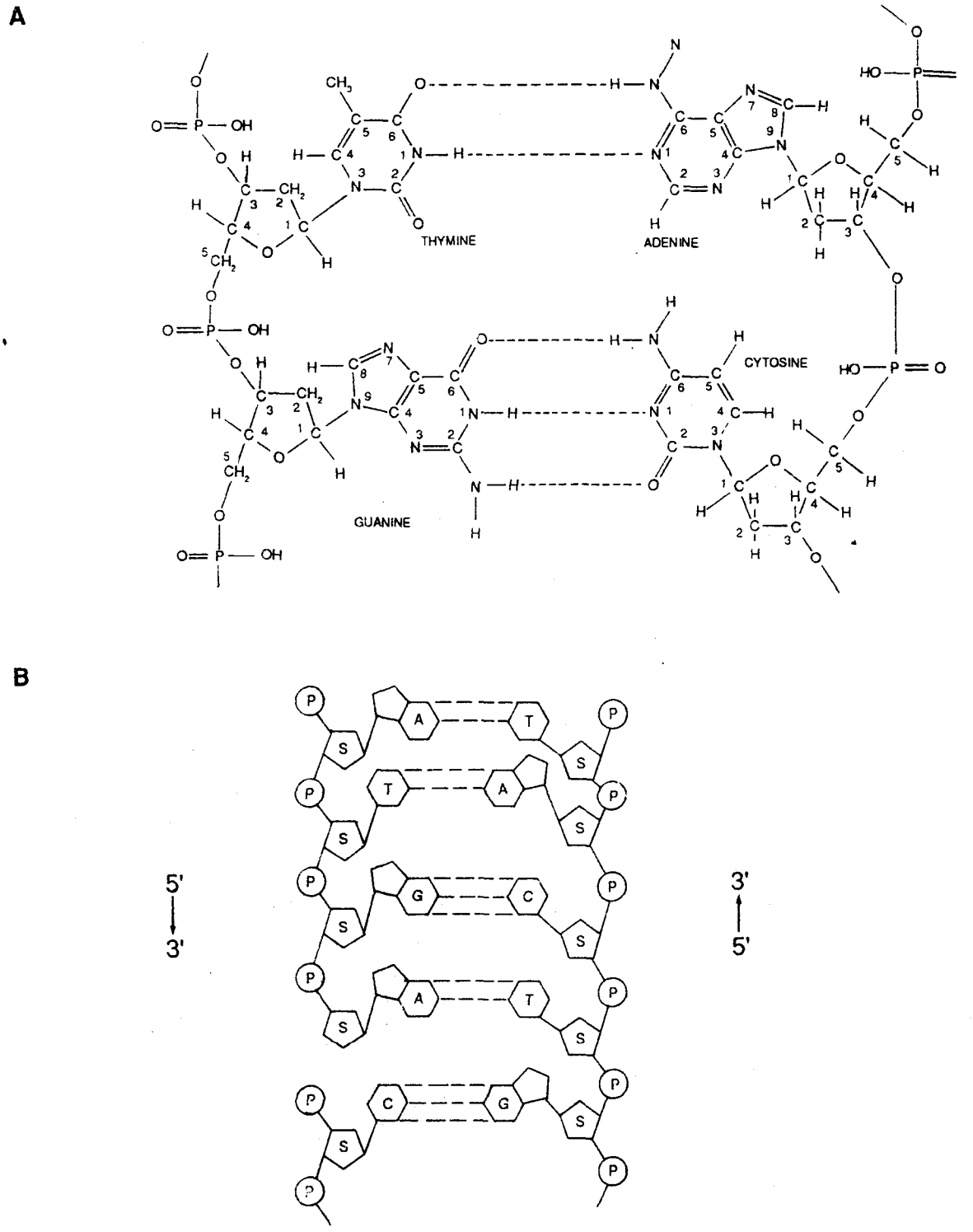

FIG. 2. DNA Structure. **A**:Chemical structure **B**:A simplified representation indicating polarity.

Fig. 2). Both strands are coiled in the same direction, so they cannot be separated without unwinding. Minor bases present in mammalian DNA include 6-methyl adenine and also 5-methyl cytosine, which is found throughout the human genome but is often concentrated in areas of heterochromatin, such as in chromosomes 1, 9, 15, 16, and Y.

Prior to cell division, new DNA must be synthesized with great fidelity. This is accomplished by separation of the two strands so that each acts as a template for the assembly of a complementary strand (see Fig. 3). Thus, two identical copies of the original DNA are produced, each composed of one original strand and one newly synthesized strand (semiconservative replication). This mechanism for producing a faithful copy of the genetic information for each daughter cell is fundamental to the BrdU and tritiated thymidine techniques discussed in subsequent chapters.

Of the four bases, two are purines (A and G), and two are pyrimidines (T and C). The precise replication of DNA is possible because the pairing of bases is specific: A pairs with T, and G pairs with C. Thus, the sequence of bases in one strand specifies the bases and their order in the complementary strand.

The way DNA stores information was elucidated in the 1960s (23,24). The genetic code consists of three bases per "codeword," so one triplet, or codon, codes for one amino acid (Table 1). A gene, then, can be understood as a linear arrangement of codons giving the instructions for the building of a protein with specific amino acids in a particular order.

TABLE 1. *The genetic code*[a]

DNA triplet	RNA triplet	Amino acid
AAA	UUU	Phenylalanine
AAT	UUA	Leucine
TAA	AUU	Isoleucine
TAC	AUG	Methionine (start)
AGA	UCU	Serine
GGA	CCU	Proline
TGA	ACU	Threonine
CGA	GCU	Alanine
ATA	UAU	Tyrosine
ATT	UAA	(stop)
GTA	CAU	Histidine
GTT	CAA	Glutamine
TTA	AAU	Asparagine
TTT	AAA	Lysine
CTA	GAU	Aspartic acid
CTT	GAA	Glutamic acid
ACA	UGU	Cysteine
ACT	UGA	(stop)
ACC	UGG	Tryptophan
GCA	CGU	Arginine
CCA	GGU	Glycine

[a] The nucleotide triplet in DNA specifies a triplet in RNA, which specifies an amino acid (or a start or stop signal). The code is "degenerate" in that each codon is not unique; for instance, UUA, UUG, CUU, CUC, CUA, and CUG all specify the amino acid leucine. A = adenine; C = cytosine; G = guanine; T = thymine; U = uracil.

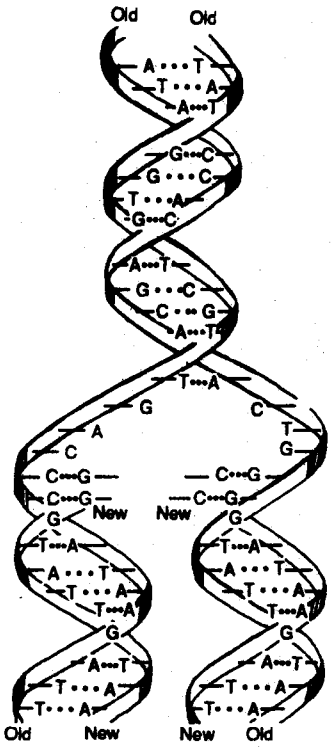

FIG. 3. DNA replication illustrating two new helices being replicated semiconservatively. (Adapted from ref. 26a.)

It was later discovered that in higher eukaryotes, the coding instructions in a gene are often interrupted by DNA sequences that are not present in the messenger RNA and are not translated into amino acids in that gene's protein. These interrupting sequences are called introns, and the sequences present in the mRNA that usually code for protein are called exons (7).

The bases in DNA are flat molecules that can stack on top of one another. The double-helical nature of DNA is maintained by these stacking forces and by the hydrogen bonds between the bases. High temperatures or high pH conditions break the hydrogen bonds, and the double-stranded helix unwinds, or denatures, into two single-stranded helices. Because G-C pairs have three hydrogen bonds and A-T pairs have only two, the A-T pairs tend to be more unstable, denaturing before the G-C pairs. Therefore, the temperature (T_m) at which a given DNA will be half denatured, or melted, is used as an index of the amount of G and C in that DNA. The curve of the rate at which this denatured DNA renatures (becomes double-helical once more) is called its Cot curve. This curve yields other information about the DNA, such as how many

sequences are present in multiple copies (repetitive DNA) versus how many are unique. Another measure of the G-C content is the buoyant density of the DNA. This is measured by forming gradients of concentration (and therefore of density) in cesium chloride during centrifugation. The DNA will collect at the band where the gradient density is equal to the DNA density. This buoyant density depends upon DNA strandedness and base composition.

Recombinant DNA Techniques

A breakthrough in the study of DNA occurred when bacterial enzymes that cut DNA at specific sequences were discovered (28). These restriction enzymes often make staggered cuts in the two DNA strands, leaving short, single-stranded tails on the ends of both fragments. These single-stranded ends easily bind to complementary fragments by base pairing. Two fragments that have attached in this way can be permanently joined by adding DNA ligase, thereby producing a recombinant molecule.

Further advances in this field took advantage of the fact that many bacteria contain plasmids—tiny circular DNA molecules—and that these plasmids can replicate autonomously in bacteria. Plasmids from the bacterium *Escherichia coli* (*E. coli*), which had only one recognition site for the restriction enzyme *Eco*RI, were cut by the enzyme; then foreign DNA, also cut with *Eco*RI, could be spliced in, and the plasmids could be sealed with ligase. The hybrid plasmids were then transferred back into *E. coli*, where they carried out the instructions of the inserted DNA and reproduced with the bacteria's own DNA (2). Using these techniques, researchers were able to isolate bacteria that had acquired a gene of interest and to make an enormous number of copies (cloning), owing to the rapid reproductive rate of bacteria (25).

Other advances in the study of DNA and genetics were made when researchers devised methods for determining the base sequence of a given DNA molecule (20,26). Investigators were later able to synthesize DNA of a desired sequence.

Because restriction enzymes cut DNA at specific nucleotide sequences or recognition sites, the length of each fragment produced depends on the distance from one recognition site to the next. Harmless natural variations exist, such as the one at a point about 7,000 nucleotides away from the beta-globin gene on chromosome 11. A recognition site for the restriction enzyme *Hpa*I is present at that point in the DNA of some people, but not others. If the site is present, a short fragment containing the beta-globin gene, 7,600 base pairs long, is produced. If absent, the beta-globin–containing fragment is 13,000 base pairs long. These normal variations have been given the name restriction fragment length polymorphisms (RFLPs).

Such fragments can be separated by electrophoresis, a process in which the DNA fragments move through a porous agarose gel that has an electric field. Smaller fragments migrate more quickly, so fragment sizes can be determined by their positions in the gel. Studies showed that in a majority of cases, the 13,000 base pair (bp) fragment was associated with sickle cell disease (27).

Direct detection of the sickle cell globin gene has been demonstrated using another restriction enzyme (*Mst*II), which cuts within the beta-globin gene as well as many other places. In the normal beta-globin gene, *Mst*II cuts at the sequence CCTGAGG, producing two fragments of 1,150 and 200 base pairs. The sickle cell mutation changes the sequence to CCTGTGG, thereby eliminating the recognition site, so *Mst*II produces a 1,350 bp fragment (28). Detecting whether a person has the two smaller fragments (normal) or the single 1,350 bp fragment is complicated by the fact that there are so many fragments of similar sizes. This is overcome by using radioactively labeled probes in a technique called Southern blotting (see Fig. 4).

A probe is a short sequence of purified DNA that is complementary to the DNA in question. In order for the probe to attach, the double-stranded DNA must be denatured by heat or alkali. The probe used to detect the sickle cell gene is a fragment of the cloned beta-globin gene, made radioactive so that it can be detected in the presence of large amounts of other DNA.

DNA to be tested for the sickle cell mutation is cut with *Mst*II. The resulting fragments are then separated by agarose gel electrophoresis and treated chemically to denature them. Next, the fragments are transferred (blotted) onto a nitrocellulose filter, to which they become bound. The transfer retains the pattern of fragments that was produced in the agarose gel. Next, the filter is exposed to the radioactively labeled probe. Once hybridized to its complementary DNA, the radioactivity can be detected by placing the filter next to x-ray film (27,28).

If a band corresponding to a DNA fragment of 1,350 base pairs shows up on the autoradiograph, it represents the sickle cell gene. If two bands of 1,150 and 200 bp appear, they represent the normal gene. If bands representing both the longer and the two shorter fragments are present, the individual has inherited the sickle cell gene from one parent and the normal beta-globin gene from the other parent and is a carrier for the sickle cell trait (28).

Because it is unusual for a genetic mutation to exist in a restriction site, other means of detection must be used. One option is to use synthetic oligonucleotides (*oligo* = few). These short molecules are engineered to match portions of a normal gene exactly. If there is

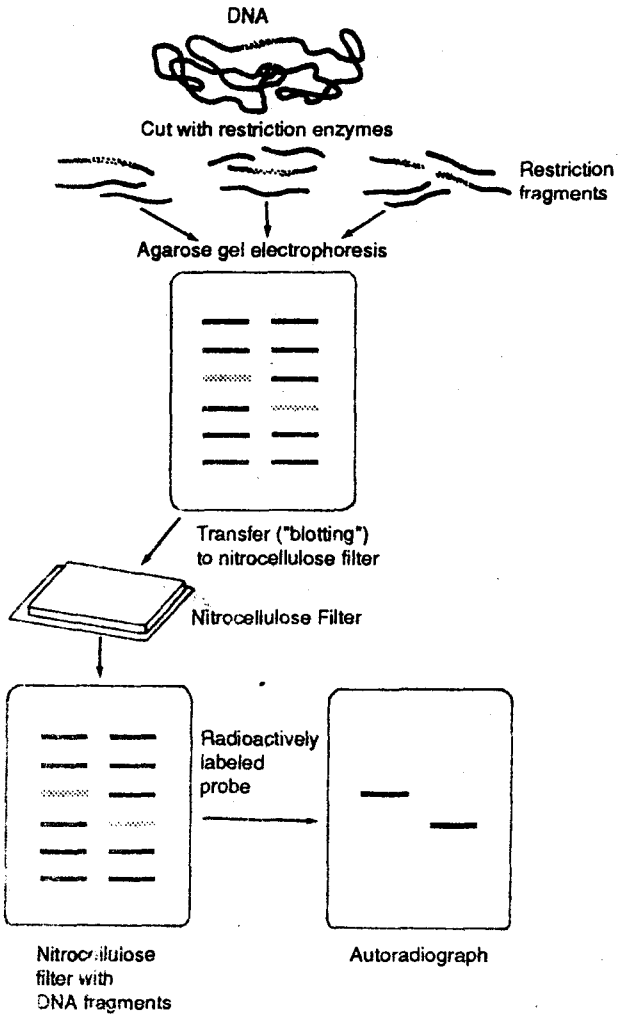

FIG. 4. Southern Blotting. DNA fragments are separated according to size by agarose gel electrophoresis. They are then transferred to a nitrocellulose filter where they are exposed to radioactive probes which hybridize with complementary sequences. The radioactive signals are detected by autoradiography using X-ray film. (Adapted from ref 28.)

a change in just one base, the hybrid molecule will be unstable and will denature easily. These oligonucleotide probes can therefore be used to detect genetic defects that involve a point mutation or change in a single base (27,28).

Another useful tool available to molecular geneticists is the polymerase chain reaction (PCR), which allows small amounts of DNA or RNA to be amplified, producing millions, even billions, of copies (29). This makes it possible to make from tiny samples of DNA amounts great enough to be analyzed using restriction enzymes, oligonucleotides, or direct sequencing.

A vast body of knowledge of DNA and its genetic implications now exists. References 2, 25, 26, 27, and 28 provide more information.

RNA

Like DNA, RNA is a polymer of ribonucleotides linked by 3′-5′ phosphodiester bonds. RNA differs from DNA in three respects: Its ribose sugar has a 2′-H group instead of 2′-OH group; it is single-stranded, rather than double-stranded; and it substitutes the base uracil for thymine to pair with adenine. DNA does not specify a protein directly; rather, a gene is expressed through an intermediary molecule, messenger RNA (mRNA). Transcription, or mRNA synthesis, uses one strand of the DNA as a template for a complementary strand of RNA (Fig. 5). After transcription, introns are spliced out and the mRNA molecule moves out of the nucleus to the cytoplasm, where it directs the synthesis of protein in the presence of ribosomes. Transfer RNA (tRNA) binds the appropriate amino acid to its anticodon, a base triplet complementary to a codon in messenger RNA. Ribosomal RNA assists in the actual protein synthesis, in which the anticodons of the tRNA molecules bind the codons of the mRNA molecule so that the attached amino acids are covalently linked in the proper linear order.

Chromosomal Proteins

The two main categories of chromosome proteins are histones and nonhistones. Interphase chromatin contains mostly histone proteins, which are characterized by their basic pH and large numbers of the amino acids arginine and lysine. Their isoelectric points (pHs at which the average charge of the molecule is zero) are always more than 10. Proteins with an isoelectric point less than 10 are classified as nonhistone proteins (NHPs). NHPs tend to be acidic, although their isoelectric points vary from 4 to 9, and are a mixture of proteins with different structural, enzymatic, and regulatory functions.

Histones

There are five major histone classes: lysine-rich H1, slightly lysine-rich H2A and H2B, and arginine-rich H3 and H4. H5 is a special histone that replaces H1 in nucleated erythrocytes. (In mature sperm, histones are replaced by a group of low molecular weight, highly basic proteins called protamines.) H3 and H4 are highly conserved in evolution, and their functions may be the same in all eukaryotes (19). Histones are extracted from chromatin by dilute acids or by high-molarity salt solutions. Acetic acid and methanol, com-

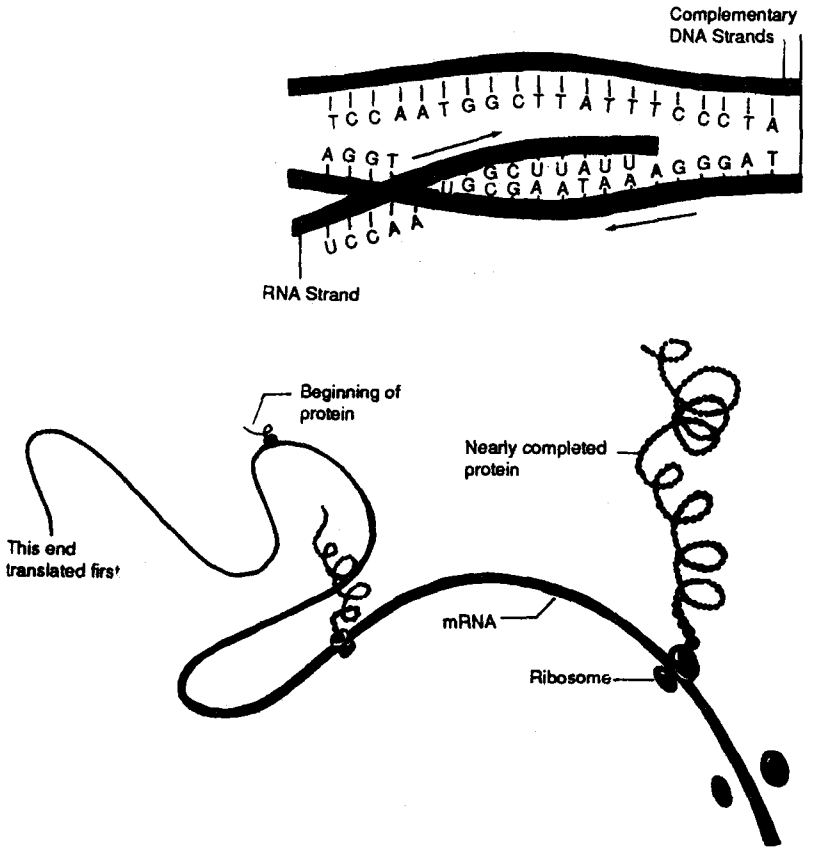

FIG. 5. DNA Transcription. Synthesis of a RNA molecule complementary to one strand of the DNA. RNA Translation. Synthesis of protein molecules specified by the RNA sequence. (From ref. 28, with permission.)

monly used to fix chromosomes, dissolve out some, if not most, of the histones. The ratio of histones to DNA in chromatin is 1:1 by weight. Histones are highly conserved in organisms from one tissue to another and between species, so cows and peas have virtually the same histones.

Nonhistones

There are several hundred nonhistone proteins (12). The nonhistones include all proteins of chromatin other than histones and are thought to be more numerous and more variable, though they make up much less of the chromatin mass (19). The nonhistone proteins are involved in chromosomal metabolism (22), in gene expression, and in higher order structure.

DNA, Proteins, and Chromatin

Histones H2A, H2B, H3, and H4 form octomers containing two molecules of each histone, giving rise to a 10 nm sphere or disk (visible with the electron microscope) called a nu-body, or nucleosome. The fifth histone, H1, is implicated in the linking and compaction of these nucleosomes. The nucleosome appears to be the basic unit of eukaryotic chromatin; it is present in dispersed or condensed chromatin, in repetitive areas of unique sequences, and in interphase and metaphase. It is associated with roughly 140 base pairs of DNA, which is wound twice around the spherical nucleosome. (For reference, an average structural gene is approximately 1,200 base pairs, which would span about six nucleosomes). When chromatin is extended by the removal of H1 histones, a linker region of about 60 base pairs of DNA can be seen between nucleosomes. With this region uncoiled, the nucleosomes are seen located along the naked DNA like beads on a string. The H1 protein is responsible for condensing these beads into a 10 nm fiber. This is coiled again into the 25 nm strands, which look lumpy or kinky under the electron microscope (see Fig. 6).

Euchromatin and Heterochromatin

Heteropyknosis is the property of chromatin of showing variations in staining intensity owing to differences in the degree of coiling or condensation of the chromatin filaments. During the cell cycle, the chromosomes condense and decondense, with maximum