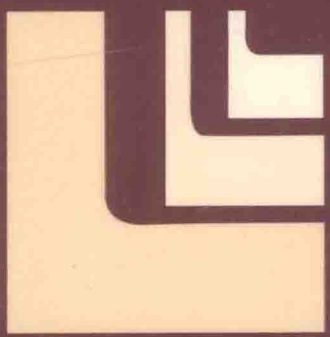
Molecular Basis of Medical Cell Biology

Gerald M. Fuller
Dennis Shields



a LANGE medical book

Molecular Basis of Medical Cell Biology

First Edition

By

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To our families:

Mark, Kevin, and Tracy Fuller

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Preface

In the past 20 years, the knowledge gained from molecular and cell biology has completely revolutionized our way of thinking about how cells work and our ability to manipulate their function. Recombinant DNA technology—in concert with the Genome Project, which is enabling us to determine the complete DNA sequences of bacteria, yeast, and mammals, including humans—is changing our understanding of genetic diseases. Native and modified genes can now be readily introduced into different cells, including the embryos of flies (*Drosophila melanogaster*), worms (*Caenorhabditis elegans*), frogs (*Xenopus laevis*), and mice (*Mus* spp.), and the effects of these mutations on individual cells or indeed on the development of the whole organism can be analyzed in cellular and molecular detail. As we approach the new millennium, the introduction of new genes into organisms has profound implications for the physician, because our ability to understand, diagnose, and treat diseases has changed fundamentally.

We are on the verge of introducing genes into cells to correct defective genetic functions and to target specific drugs to cancer cells, thereby killing only those cells whose growth is uncontrolled. Furthermore, our knowledge of the complex signaling networks and interactions that regulate cell growth and development has expanded enormously in the past five years. These discoveries are leading to new diagnostics and treatment modalities for disorders—such as cancer, diabetes, cardiac disease, and genetic defects—that were previously inconceivable or were confined to the realms of science fiction. To take full advantage of the new therapeutic interventions, the modern physician must understand the molecular details of cell function, communication, development, and growth.

The aim of this book is to present these details in a precise, straightforward, and succinct fashion, which will enable medical students, physicians, and advanced undergraduates to fully understand the cellular and molecular basis of disease processes. To achieve this goal, we have attempted to describe mammalian cells in terms of their molecular architecture and biochemical function. As each organelle system is described, we have related its function to specific diseases. For example, we now have a detailed molecular and cellular understanding of cystic fibrosis, a hereditary disease affecting approximately 1 in 2000 individuals. In this disease, a complex membrane protein that normally resides on the plasma membrane and functions in chloride transport has a single amino acid change. This alteration dramatically affects the protein's cellular localization because the mutated protein cannot fold correctly in the endoplasmic reticulum (ER). Consequently, instead of its normal transport to the cell membrane, the protein is retained in the ER and degraded in the cytoplasm.

We have attempted to integrate such knowledge derived from recent research exploiting such diverse organisms as yeast cells, fruit flies, nematodes, and mice to present a comprehensive overview of modern cell biology as it relates to understanding human disease. In the early chapters, we discuss the fundamental principles of cell organization and the basic molecules that constitute organelles. In the later chapters, we discuss the function of individual organelles, the organization of the cytoskeleton, intracellular protein transport and secretion, signal transduction, cell signaling, and growth regulation. Although each chapter builds on previous material, we have tried to ensure that chapters can be readily understood on their own; thus each one presents a comprehensive overview of the material that will enable readers to rapidly review individual topics. This book will therefore be a valuable aid to students preparing for medical board examinations.

The idea of the book was developed from our being course leaders in cell biology at our respective universities. We felt that most medical students would have had more than a "smattering" of cell biology, and indeed many have been exposed to more comprehensive text than ours. Our goal is to refresh in students' memories the concepts of modern cell biology and to

show how these concepts apply to cell function in a medical or disease context. We hope we have done this. Many important molecular details had to be glossed over in keeping with the original plan of having a text that could be revised frequently and integrated into the teaching of histology at an affordable price.

ACKNOWLEDGMENTS

We wish to acknowledge the generous contributions from other authors and their texts. We are especially appreciative of the generosity of several of the Appleton & Lange authors: Drs. Walter X. Balcavage, William F. Ganong, Eric R. Kandel, Robert K. Murray, and their respective coauthors. In addition, we acknowledge the aid of two of the gold standard texts of modern cell biology, *Molecular Biology of the Cell*, by Dr. Bruce Alberts and colleagues, and *Molecular Cell Biology,* by Dr. Harvey Lodish and coauthors. The textual organization of these books contributed much to the way we put our chapters together.

On a personal note we wish to acknowledge very special individuals who helped each of us in countless ways. Mr. Nelson L. Fuentes, at the University of Alabama, and Ms. Elyse Rizzo, at Albert Einstein College of Medicine, were especially important in keeping the authors on track and in organizing each of the chapters. We also thank Ms. Amanda Suver for taking on the task of editing and working through some difficult administrative problems.

Gerald M. Fuller, PhD
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Birmingham
September, 1997

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THE EVOLUTION OF A SCIENTIFIC DISCIPLINE

Early cell biologists referred to themselves as **cytologists.** These scientists were mostly concerned with cell structure (morphology), and their discipline focused on sectioning tissues and staining tissues and cells. They examined the structures within cells by using different types of stains and fixation embedding procedures and then extrapolated the function of the cells by observing their structure. This approach was remarkably accurate for the time, but as the tools and techniques changed so did the cell biologist. Cytologists and microanatomists became **cell biochemists** and **cell molecular biologists.** Physical and molecular biochemistry led the way to understanding the three-dimensional conformation of molecules that control cell activity. The application of electron beams in the development of the electron microscope became as important in creating images of cells as were light waves. As techniques in immunology developed and the understanding of how an antibody recognized its antigen increased, cell biologists found that antibodies could pinpoint molecules within a cell. The use of antibodies has allowed the cell biologist to probe the interior of the cell in ways never before thought possible. Today, therefore, cell biologists use antibodies to localize, quantitate, and determine the interactions of even tiny amounts of a protein within cellular compartments. It is difficult to judge any one major leap in biochemistry, genetics, immunology, embryology, or physical chemistry as the most crucial technological advance in cell biology. If there were one achievement equal to the microscope (of any variety), however, it might be the use of antibodies to identify, localize, and follow the fate of regulatory proteins within the cell.

A new horizon in cell biology emerged when it was shown that individual types of mammalian cells could be grown in the laboratory with a technique referred to as **tissue and cell culture.** This technology has been used to grow cells from all organs of the body, including skin, liver, kidney, pituitary glands, muscle, and even some types of cells from the nervous system. The development of this technology has led the way to the design of experiments that mimic environmental conditions of the cell. With the use of cell culture technology, normal physiologic conditions of the body can be subtly altered. The responses of the cell may therefore be examined in ways that were impossible with cells still in the animal. Step by step, cell type by cell type, new information has been gathered about how cells communicate with one another. The importance of **extracellular matrix molecules** to cell growth and communication has been firmly established through the use of cell culture systems. Information about cell division, the molecules that control it, cell aging, and differences between tumor cells and normal cells has begun to unfold. By combining antibodies with cells on a tissue culture plate, cell biologists have been able to define molecules that were missing, defective, or overactive during changes in cell "social behavior."

Since the late 1970s, **molecular biology** has provided the cell biologist with powerful tools to explore the chemistry and regulation of individual genes and has completely changed how a cell can be studied. Cloning, sequencing, manipulating, and expressing genes in tissue culture cells are as routine today as making specific antibodies was only a decade ago. Now, so we can fully understand how a protein functions, it is usually cloned and then made in huge quantities with the use of prokaryotic or eukaryotic cells. The chemistry of the protein, including its three-dimensional configuration, can therefore be examined in detail. Any individual molecule can be isolated, and its innermost structure determined. Not only can one make a rare molecule in amounts never before possible, but the molecule can be genetically inserted into a cell and the response to its "overexpression" examined. By knowing exactly which molecules are involved in a cell function, the cell biologist can take the function apart, molecule by molecule, and determine the details of each interaction in a way never even dreamed of only 2 or 3

decades ago. Further, for the past 10 years it has been possible to introduce genes into mice—the so-called transgenic mice—and study their function. This technology has paved the way for creating animals that manifest a particular genetic defect and studying its phenotype during development as well as in adult animals. In addition, by targeting particular oncogenes to specific cells, it has been possible to produce tumors in these cells that can then be studied both in the whole animal as well as in tissue culture.

We would indeed be remiss if we failed to emphasize how much has been learned of human cell function by studying less complex organisms. We cite here four different types of organisms and how each has been especially useful. Our list, however, is by no means exhaustive. First, yeast cells have been especially useful in elucidating the molecular events of the cell cycle because they can be genetically manipulated and easily grown in the laboratory. The **kinases** and **cyclins,** which are subunits of proteins whose synthesis and degradation increase and decrease during the cell cycle, are the major molecules that drive a cell through the cycle. The study of these molecules has been instrumental in developing our current knowledge of how mammalian cells progress through the cell cycle. Yeast cells have also been a major cellular model for advancing our understanding of the molecular basis of secretion: the pathways of intracellular vesicle trafficking, protein sorting, and organelle biogenesis are now understood in some detail as a result of using yeast cells.

Two completely different invertebrate organisms, *Caenorhabditis elegans* (a small nematode) and *Drosophila melanogaster* (the fruit fly), have served as powerful models for studying cell development, organogenesis, programmed cell death, and the molecular genetics of cell behavior. Informed physicians will immediately recognize the importance of new information about cell function, derived from the nematode or fruit fly, and how it could easily have a direct bearing on their understanding of human cellular behavior. Indeed, the importance of fruit flies in contributing to our understanding of early development and embryogenesis was recently recognized by the awarding of the Nobel Prize to Christine Nusslein-Volhard, Eric Wieschaus, and Edward Lewis (1995).

In summary, all eukaryotic cells use similar molecules to carry out cell function; thus, we are all related and likely share common origins. The greater our understanding of simple systems, the more we know about ourselves.

MAMMALIAN CELL PHENOTYPES

Although not all mammalian cells look or behave alike, they share identical genetic material. We now know that the 200 or so different cellular phenotypes within the human body are determined by which genes are expressed and by the signals that control when a particular gene or set of genes is expressed. In this chapter, we discuss the many different cellular phenotypes in the mammalian body and how they are socially and functionally arranged. We then turn our attention to the basic mechanisms that control gene expression and the locations in the cell that are most sensitive to regulatory events. Finally, we consider how cells in adult tissue replenish themselves during adult life.

The Systematic Organization of Cells in Tissues

The term **tissue** (L. *texere,* to weave) was coined by the French surgeon Bichat to describe his concept of the different "textures" of the human body. On the basis of his extensive studies of cadavers, Bichat proposed that the body is composed of many different materials woven together to form a variety of fabrics or tissues. His observations were indeed correct. It was later recognized, however, that there are actually only four basic types of cells: epithelial, connective, muscle, and nervous. Within each of these basic tissues are many subtypes that exhibit different morphologic and functional phenotypes.

We do not describe here the details of each basic tissue; that task is properly reserved for histology. It is valuable, however, to describe briefly the characteristics of each fundamental cell type before discussing the molecular events that occur within these cells.

Cells of the epithelium. The exterior of the body and nearly all the internal surfaces are covered by a continuous sheet of cells known as the epithelial membrane, which is composed of epithelial cells. This tissue exhibits close cell-to-cell contact and forms strong, tight connections that are impervious to the passage of fluids (Figure 1–1). Epithelial cells are polarized, as they have distinct surfaces (apical and basolateral). These cells also have a broad range of specializations, and one of their more prominent features is the propensity to secrete fluids produced by the cell. These substances can be directly secreted to the cell surface, or the epithelial cells can form ducts or channels through which copious amounts of material are released for lubrication. Certain types of epithelial cells secrete directly into the blood stream; these cells are usually located near capillary networks (see Chapter 2).

Cells of connective tissue. The most basic role of connective tissue cells is to support, connect, and nourish cells in other tissues. Many cell types within this category produce substantial quantities of extracellular matrix material (Figure 1–2). The organization of this matrix is proteinaceous, as it contains various types of collagens and other structural proteins, such as fibronectin, laminin, and vitronectin (see Chapter 8). One of the more obvious specializations of connective tissue is that it produces unique types of

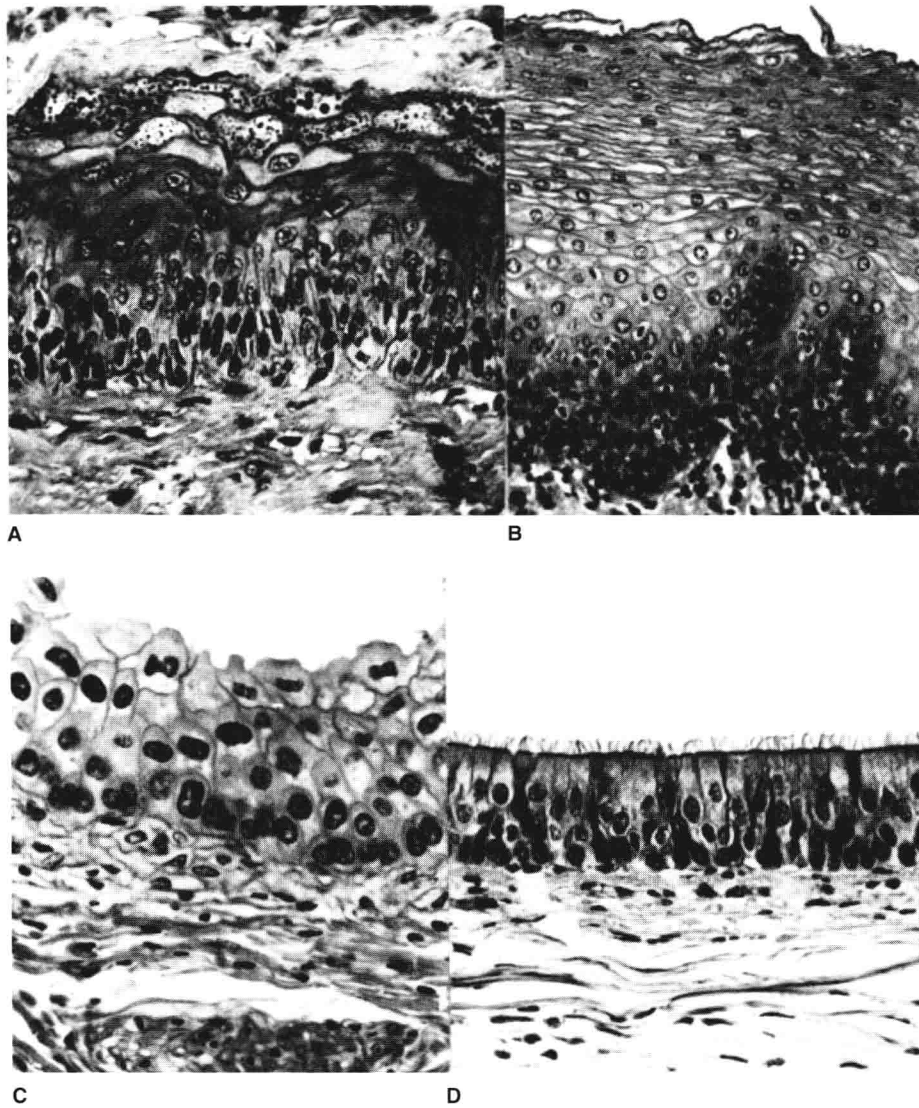

Figure 1–1. Types of epithelial tissue. These photomicrographs show four types of complex epithelial tissue: stratified squamous keratinized epithelium (A), stratified squamous nonkeratinized epithelium (B), transitional epithelium (C), and ciliated pseudostratified columnar epithelium (D). All slides were stained with hematoxylin and eosin. × 500. (From Junqueira LC: *Basic Histology*, 8th ed. Appleton & Lange, 1995, p. 62, with permission.)

collagen and other matrix molecules. One important structural element of connective tissue cells is the **basement membrane;** most epithelial cells are supported on this structure. Although the basement membrane is mostly composed of materials derived from connective tissue cells, other cell types contribute to its structure in certain instances (Figure 1–3).

The **fibroblast** is a relatively undifferentiated connective tissue cell. It is a precursor cell that forms other types of connective tissue cells, including adipocytes (fat cells), smooth muscle cells, and bone- and cartilage-producing cells. Certain fibroblastic cells of connective tissue specialize in the production, modification, and remodeling of bone and cartilage. These cells are known as osteoblasts, osteocytes, chondroblasts, and chondrocytes. The manufacture of bone and cartilage is a dynamic process that typically occurs throughout the lifetime of an organism. Blood cells, including erythrocytes, monocytes, neutrophils, basophils, eosinophils, and platelets, are derived from

Figure 1–2. Connective tissue matrix. In an electron micrograph, various elements of the matrix are visualized. The matrix consists of proteins and cells embedded in ground substance. Collagen (C) and elastic (E) fibers and fibroblast cells (F) are visible. The granularity of the ground substance is an artifact of the fixation procedure used in preparing the sample. × 100,000. (From Junqueira LC: *Basic Histology,* 8th ed. Appleton & Lange, 1995, p. 89, with permission.)

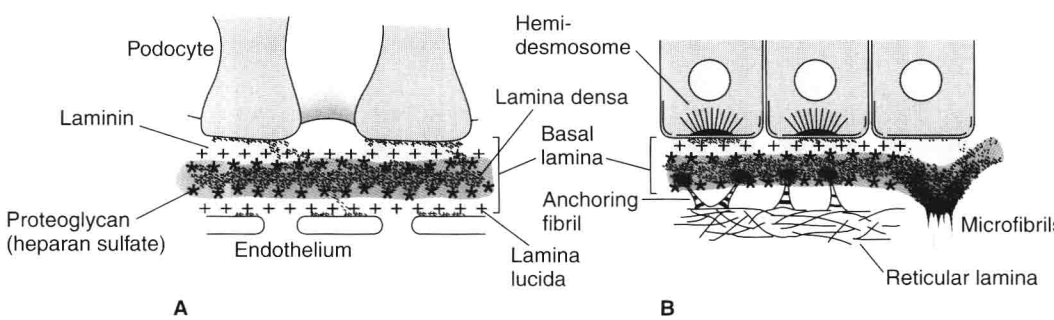

Figure 1–3. Types of basement membranes. This thick basement membrane (A) connects an epithelial and an endothelial cell layer. It is formed by fusion of the basal laminae of the two layers. A thick central lamina densa (darker colored zone) has a lamina lucida (lighter colored zone) on either side. Such basement membranes are found in the kidney glomerulus (shown here) and in the pulmonary alveoli. The more common type of basement membrane (B) forms the boundary between an epithelial cell layer and connective tissue. It is formed by association of the cell's basal lamina and the reticular lamina of the connective tissue. Note anchoring fibrils composed of type VII collagen, which bind the basal lamina to collagen. Microfibrils perforate the basal lamina, binding it to elastic fibers in connective tissue. (From Junqueira LC: *Basic Histology,* 8th ed. Appleton & Lange, 1995, p. 63, with permission.)

connective tissue cells. These cells are produced in the myeloid tissue of the inner compartment of bones, called the bone marrow.

Cells of muscle tissue. The third basic tissue is muscle. Although cells in this tissue are specialized for contraction, their outward appearance is usually quite distinct (and dissimilar from one another). There are four categories of contractile cells: skeletal or striated muscle cells, cardiac (heart) muscle cells, smooth muscle cells (derived from fibroblasts), and myoepithelial cells (derived from ectoderm).

Skeletal muscle cells usually have a highly elongated shape and are often referred to as muscle fibers. A single muscle fiber (cell) is a syncytium that contains many nuclei with a common cytoplasm. New muscle fibers develop from the fusion of myoblasts (Figures 1–4 and 1–5). Cardiac muscle cells, like striated muscle cells, form a syncytial structure. The contractile proteins (myosin, actin, and other structural proteins) are aligned in an orderly arrangement called a **sarcomere** (see Chapter 7). Smooth muscle cells do not form syncytial connections. Rather, they align into long bundles, and their contraction is much slower and more prolonged than that of striated or cardiac cells. These cells, which are important components of blood vessels, participate in directing blood flow, especially in the microcirculation (capillary beds). Myoepithelial cells also lack striations and are derived embryologically from ectoderm rather than from mesoderm, which is the embryonic origin of the other muscle cells. These contractile cells regulate the responses of certain sensory cells, such as the iris, sweat glands, milk, and other glands that respond to sensory information (Figure 1–6).

Cells of nervous tissue. Nerve cells, the fourth major class of basic tissue, are characterized by their "irritability" and capacity to conduct electrical impulses. These cells constitute the master communication network of the body. The neuron is divided into three large groups: unipolar, bipolar, and multipolar. These groups reflect the number of processes that arise from the cell body (Figure 1–7). The simplest,

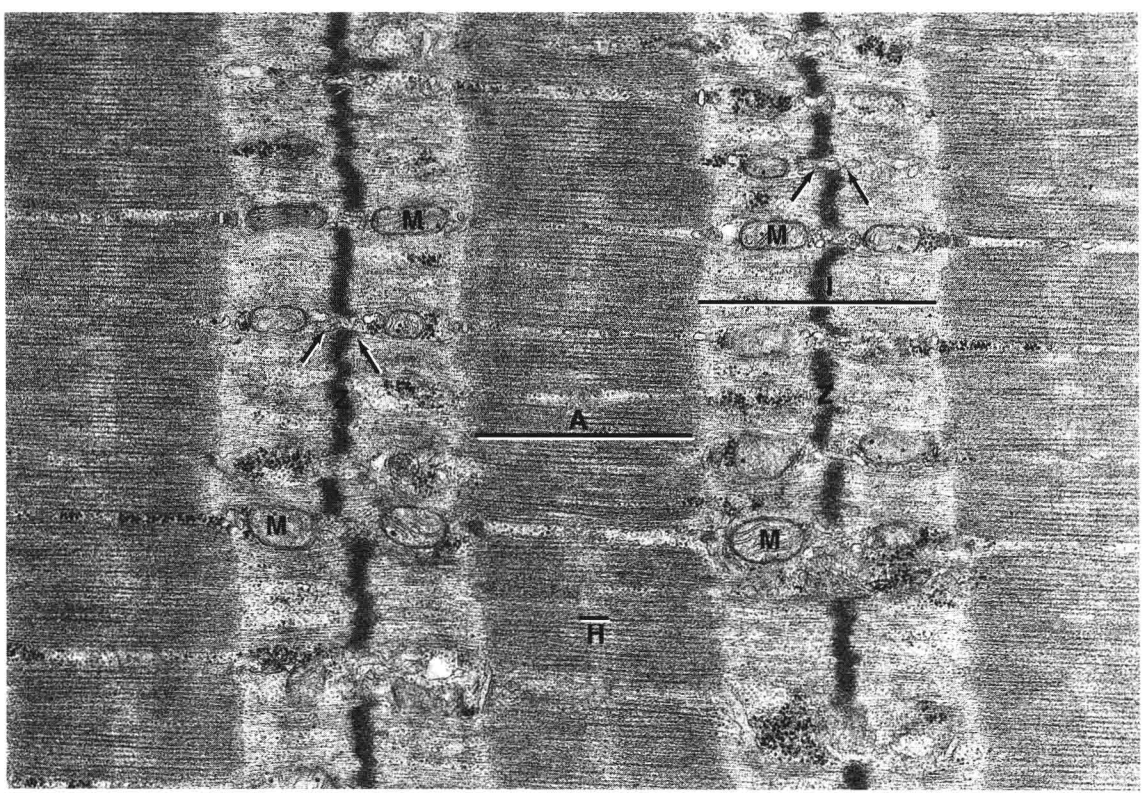

Figure 1–4. Skeletal muscle fiber. This electron micrograph shows the alignment of myofibrils that results in the characteristic striated morphology of this type of cell. The sarcomere, the contractile unit of the fiber, is delineated by adjacent Z lines (Z), which bisect the I band (I). Within the sarcomere is an electron-dense A band (A) bisected by an electron-lucent H band (H). Mitochondria (M) are also visible. Rat; × 24,280. (Reproduced, with permission, from Berman I: *Color Atlas of Basic Histology*. Appleton & Lange, 1993, p. 72. Courtesy of M Snow.) See also Figure 7–12 for additional details.

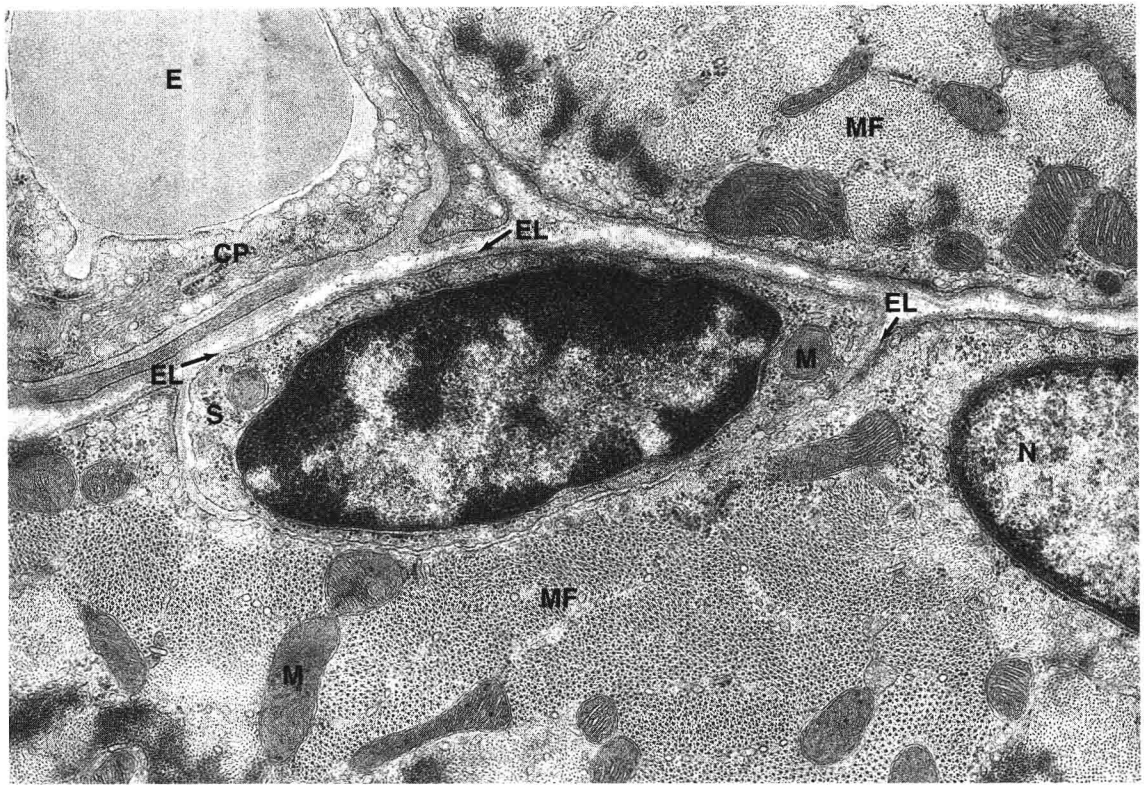

Figure 1–5. Skeletal muscle satellite cell in cross section. This electron micrograph of a skeletal muscle satellite cell shows that it (S) lies completely within the external lamina (EL) of the muscle fiber (MF) at the bottom of the micrograph. (N = nucleus of muscle fiber; M = mitochondria; CP = cell process of capillary endothelial cell; E = erythrocyte.) Rat; × 25,600. (Reproduced, with permission, from Berman I: *Color Atlas of Basic Histology*. Appleton & Lange, 1993, p. 72. Courtesy of M Snow.)

the unipolar neuron, has a single primary process with many branches; one branch serves as the axon, and the others function as dendrites. Most unipolar neurons are found in invertebrates. The bipolar neuron has two major identifiable processes: a dendritic process with many branches that convey information from the periphery to the cell body and an axon that conveys information from the cell body to other neurons. The axons of neurons terminate at neuronal junctions called **synapses.** Electrical impulses are transferred from one neuron to the next by secretion of chemical substances called **neurotransmitters,** which are released at the synaptic junction. Bipolar neurons communicate signals to the central nervous system (CNS) through neuron relay systems. Multipolar neurons are predominant in the CNS of vertebrates. These cells have a single axon, but they have a complex array of dendritic connections. For example, a multipolar axon of a spinal motor cell possesses a moderate number of dendrites, approximately 10,000. Approximately 2000 of these dendritic connections occur at the cell body, whereas approximately 8000 connections occur on the dendritic tree. A Purkinje cell in the cerebellum is a much larger multipolar neuron and has as many as 150,000 dendritic contacts.

A second major class of nervous system cells is composed of the **glial cells.** There are approximately 10–50 times more glial cells than neurons in the CNS of vertebrates. The three main types of glial cells are oligodendroglial cells, Schwann cells, and astrocytes. Glial cells perform several major functions in the CNS, including:

1. support elements for neurons and separate and insulate neurons from one another;
2. produce myelin, which is an insulating sheath necessary for certain types of neurons;
3. serve as scavenger cells to clear cellular debris;
4. help form the impervious cellular barrier between the brain and capillaries, called the blood-brain barrier.

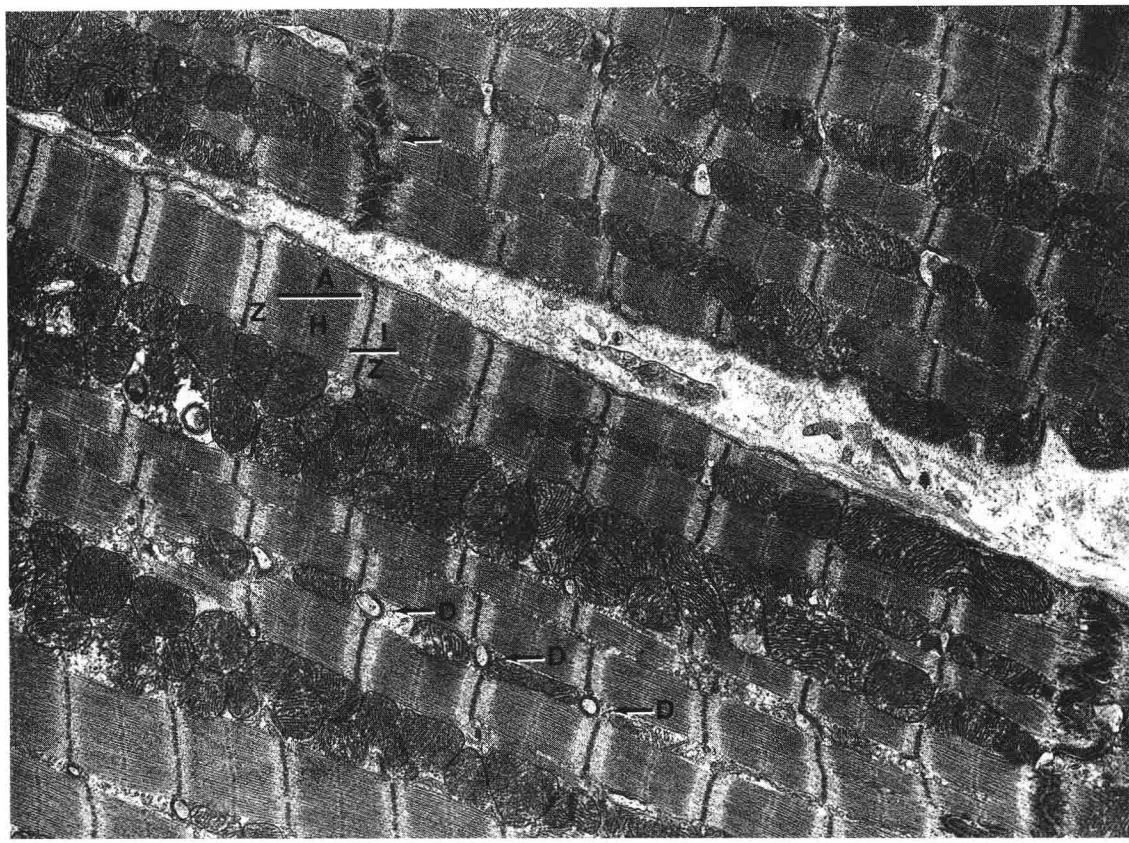

Figure 1–6. Cardiac muscle. This low-power electron micrograph of cardiac muscle illustrates its characteristic features. As in other skeletal muscle, a sarcomere is delineated by adjacent Z lines (Z). Note the intercalated disk (arrow), which is a unique feature of cardiac muscle morphology. (I = I band; A = A band; H = H band; M = mitochondria; D = diads of tubular system.) Rat; × 5500. (Reproduced, with permission, from Berman I: *Color Atlas of Basic Histology.* Appleton & Lange, 1993, p. 77. Courtesy of J-P Brunschwig.)

Glial cells participate in other important roles within the CNS, and these functions are still being discovered and documented.

Different Phenotypes of Cells of the Four Basic Tissues

We have very briefly listed and commented on the four basic types of cells that comprise the tissues of the body. Like the carbon atom of organic chemistry, these cells have been modified in almost unimaginable ways to form phenotypes far removed from the original basic structure of the progenitor cells. This same concept of building and modifying the basic structure of the cell is fundamental to the differentiation and formation of specialized function. A compendium of some 200 distinctly different cell types was recently compiled from histology texts. The cells of each category were identified and examined with the use of light and electron microscopy. This catalog of human cells has a particularly useful focus: It lets us see in a relatively small space a variety of cellular phenotypes that have been developed to carry out myriad cellular tasks (Table 1–1).

For more on the structure of cells that play a key role in human health and disease, the reader is referred to in *Color Atlas of Basic Histopathology*, by Clara Milikowski and Irwin Berman, Appleton & Lange, 1997.

REGULATION OF GENE EXPRESSION: BRIEF OVERVIEW

With very few exceptions, each cell in the human body contains precisely the same complement of genes (thought to be between 100,000 and 300,000). Cells differ so greatly from one another because different combinations of genes are expressed in