EIGHTH EDITION

BIOCHEMISTRY

Mary K. Campbell
Shawn O. Farrell

Inside:

BIOChemistry **Hot Topics Magazine!**

"Stem cells have brought tremendous hope for curing serious medical conditions while also creating a large amount of ethical controversy."

"The way stem cells work affects the whole body."

LIST OF ABBREVIATIONS

A	Adenine
ACAT	Acyl-CoA cholesterol acyl transferase
ACP	Acyl carrier protein
ADP	Adenosine diphosphate
AIDS	Acquired immunodeficiency syndrome
AMP	Adenosine monophosphate
ATCase	Aspartate transcarbamoylase
ATP	Adenosine triphosphate
bp	Base pairs
C	Cytosine
cAMP	Cyclic adenosine monophosphate
CAP	Catabolite activator protein
CDP	Cytidine diphosphate
Chl	Chlorophyll
CMP	Cytidine monophosphate
CoA (CoA-SH)	Coenzyme A
CoQ	Coenzyme Q
CTP	Cytidine triphosphate
d	Deoxy
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DV	Daily value
EF	Elongation factor
ER	Endoplasmic reticulum
FAD	Flavin adenine dinucleotide (oxidized form)
$FADH_2$	Flavin adenine dinucleotide (reduced form)
fMet	*N*-Formylmethionine
FMN	Flavin mononucleotide
G	Guanine
GDP	Guanosine diphosphate
GMP	Guanosine monophosphate
GSH	Glutathione (reduced form)
GSSG	Glutathione (oxidized form)
GTP	Guanosine triphosphate
Hb	Hemoglobin
HDL	High-density lipoprotein
HIV	Human immunodeficiency virus
HMG-CoA	β-Hydroxy-β-methylglutaryl-CoA
HPLC	High-performance liquid chromatography

IF	Initiation factor
K_M	Michaelis constant
LDL	Low-density lipoprotein
Mb	Myoglobin
NAD^+	Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
P_i	Phosphate ion
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PEP	Phosphoenolpyruvate
PIP_2	Phosphatidylinositol *bis*phosphate
PKU	Phenylketonuria
Pol	DNA polymerase
PP_i	Pyrophosphate ion
PRPP	Phosphoribosylpyrophosphate
PS	Photosystem
RF	Release factor
RFLPs	Restriction-fragment-length polymorphisms
RNA	Ribonucleic acid
RNase	Ribonuclease
mRNA	Messenger RNA
rRNA	Ribosomal RNA
tRNA	Transfer RNA
snRNP	Small nuclear ribounuclear protein
S	Svedberg unit
SCID	Severe combined immune deficiency
SSB	Single-strand binding protein
SV40	Simian virus 40
T	Thymine
TDP	Thymidine diphosphate
TMP	Thymidine monophosphate
TTP	Thymidine triphosphate
U	Uracil
UDP	Uridine diphosphate
UMP	Uridine monophosphate
UTP	Uridine triphosphate
V_{max}	Maximal velocity

BIOCHEMISTRY

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8th EDITION

Mary K. Campbell
Mount Holyoke College

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To all of those who made this text possible and especially to all of the students who will use it.

—Mary K. Campbell

To the returning adult students in my class, especially those with children and a full-time job . . . my applause.

—Shawn O. Farrell

About the Authors

Mary K. Campbell

Mary K. Campbell is professor emeritus of chemistry at Mount Holyoke College, where she taught a one-semester biochemistry course and advised undergraduates working on biochemical research projects. She frequently taught general chemistry and physical chemistry as well. At some point in her 36 years at Mount Holyoke, she taught every subfield of chemistry, except the lecture portion of organic chemistry. Her avid interest in writing led to the publication of the first seven highly successful editions of this textbook. Originally from Philadelphia, Mary received her Ph.D. from Indiana University and did postdoctoral work in biophysical chemistry at Johns Hopkins University. Her area of interest includes researching the physical chemistry of biomolecules, specifically, spectroscopic studies of protein–nucleic acid interactions.

Mary enjoys traveling and recently visited parts of Mexico near her current home in Tucson, Arizona. She participates in events at the University of Arizona and enjoys hiking in the desert and the mountains.

Shawn O. Farrell

Shawn O. Farrell grew up in northern California and received a B.S. degree in biochemistry from the University of California, Davis, where he studied carbohydrate metabolism. He completed his Ph.D. in biochemistry at Michigan State University, where he studied fatty acid metabolism. For 18 years, Shawn worked at Colorado State University teaching undergraduate biochemistry lecture and laboratory courses. Because of his interest in biochemical education, Shawn has written a number of scientific journal articles about teaching biochemistry. He is the coauthor (with Lynn E. Taylor) of *Experiments in Biochemistry: A Hands-On Approach.* Shawn became interested in biochemistry while in college because it coincided with his passion for bicycle racing. An active outdoorsman, Shawn raced competitively for 17 years and now officiates at bicycle races around the world. He is currently the technical director of USA Cycling, the national governing body of bicycle racing in the United States. He is also an avid fly fisherman, a third-degree black belt in Tae Kwon Do, and a first-degree black belt in combat hapkido. Shawn has also written articles on fly fishing for *Salmon Trout Steelheader* magazine. His other passions are soccer, chess, and foreign languages. He is fluent in Spanish and French and is currently learning German and Italian.

On his fiftieth birthday, he had his first downhill skiing lesson and now cannot get enough of it. Never tired of education, he visited CSU again, this time from the other side of the podium, and earned his Master of Business Administration in 2008.

Brief Contents

Magazine: Hot Topics in Biochemistry

Contents

Preface

This text is intended for students in any field of science or engineering who want a one-semester introduction to biochemistry but who do not intend to be biochemistry majors. Our main goal in writing this book is to make biochemistry as clear and applied as possible and to familiarize science students with the major aspects of biochemistry. For students of biology, chemistry, physics, geology, nutrition, sports physiology, and agriculture, biochemistry impacts greatly on the content of their fields, especially in the areas of medicine and biotechnology. For engineers, studying biochemistry is especially important for those who hope to enter a career in biomedical engineering or some form of biotechnology.

Students who will use this text are at an intermediate level in their studies. A beginning biology course, general chemistry, and at least one semester of organic chemistry are assumed as preparation.

What's New

All textbooks evolve to meet the interests and needs of students and instructors and to include the most current information. Several changes mark this edition.

Biochemistry Hot Topics This magazine insert features up-to-date articles on new breakthroughs and topics in the area of biochemistry such as stem cells, malaria, the breast cancer gene (BRCA), aging, happiness, and more!

Innovative and New Presentation of Biochemical Connections. Plus Many New Topics! In response to customers' demand for more Biochemical Connection boxes, we have added several new boxes to the text. See a full listing of Biochemical Connections boxes in the Table of Contents. Biochemical Connections cover a variety of important concepts and new research. They are now flowed in with the narrative and are placed exactly where they need to be read in each chapter. And although they have a different presentation than the rest of the narrative, they are meant to be read with the narrative and should not be skipped. They are like crescendos in classical music—the change in tempo from the usual narrative to the unique visual presentation and voice of the Biochemical Connections prevents the student's level of interest from dipping—students are always engaged.

New Marginal Glossary! No more flipping back and forth to read full definitions of key terms. Terms are now defined in the margins.

Updated Coverage Each chapter in the text has been updated with the current developments and scientific findings in the biochemistry field.

Table of Changes by Chapter

Chapter 1	Revised material on classification schemes based on kingdoms and domains, one new EOC exercise
Chapter 2	Expanded section on types of intermolecular forces, eight new EOC exercises
Chapter 3	Revised material on peptide hormones, eight new EOC exercises
Chapter 4	Deleted Biochemical Connections box on nutrition, added Biochemical Connections box on sickle cell anemia, six new EOC exercises
Chapter 5	Added new section on protein identification techniques, added new section on proteomics using material from previous edition's Biochemical Connections box, eight new EOC exercises
Chapter 6	Reversed order of Sections 6-5 and 6-6, expanded section on inhibition to include uncompetitive and mixed inhibition, deleted Biochemical Connections box on enzymes and memory, simplified section on Michaelis-Menten derivation, added section on kinetics with multiple substrates, added ten new EOC exercises
Chapter 7	New Biochemical Connections box on medicinal effects of a compound from the willow tree, four new EOC exercises
Chapter 8	New material on lipid composition of intracellular membranes, added material on membranes in drug delivery, discussion of tyrosine kinases as membrane receptors related to Hot Topics article on G-protein-coupled receptors, four new EOC exercises
Chapter 9	Deleted Biochemical Connections box on the DNA family tree
Chapter 10	Expanded material on the replisome, expanded material on double-stranded breaks in DNA repair, eight new EOC exercises
Chapter 11	Expanded material on operons, added material on the role of mediator in eukaryotic transcription, added material on chromatin remodeling and histone modifying enzymes, deleted Biochemical Connections box on TFIIH, expanded material on micro RNA and RNA interference, added new Biochemical Connections box on epigenetics, deleted Biochemical Connections box on transcriptional proofreading, nineteen new EOC exercises
Chapter 12	Added new Biochemical Connections box on virology and how the flu can alter the reading frame of translation, material on selenocysteine removed from a Biochemical Connections box and put into the main chapter, added new material on start codon in immune system, added material on how the ribosome is involved in protein folding, eight new EOC exercises
Chapter 13	Modified section on Flavr Savr tomatoes in Biochemical Connections box, deleted Biochemical Connections box on RNA interference
Chapter 14	Added a new section on AIDS, deleted two Biochemical Connections boxes on the flu, both of which were moved to a Hot Topics article, added a new Biochemical Connections box on vaccines, nine new EOC exercises
Chapter 15	Revised treatment of coupling of reactions in bioenergetics, six new EOC exercises
Chapter 16	Discussion of soluble vs. insoluble fiber, expanded treatment of oligosaccharides in blood type determinants, four new EOC exercises
Chapter 17	Discussion of Warburg effect and carbohydrate metabolism in cancer cells, extended discussion on hormonal control in carbohydrate metabolism, four new EOC exercises
Chapter 18	Added discussion of reciprocal regulation and hormonal control of carbohydrate metabolism, six new EOC exercises
Chapter 19	New box on use of labeling to determine the origin of CO_2 released by citric acid cycle, added material on substrate-level phosphorylation, two new EOC exercises
Chapter 20	Condensation of material of historic interest to sharpen focus of discussion of electron transport chain, three new EOC exercises
Chapter 21	New material on hormonal control of appetite, five new EOC exercises
Chapter 22	Updated box on anti-malarial plants to correlate with Hot Topics article on malaria, updated box on chloroplast genes to include epigenetic mechanisms of control
Chapter 23	New material on possible roles of *S*-adenosylmethionine in the nitrogenase reaction and in histone methylation
Chapter 24	Expanded material on obesity, added Biochemical Connections box on obesity, diabetes, and cancer, deleted Biochemical Connections box on aging (moved to Hot Topics), six new EOC exercises

New Design and Enhanced Labeling in Art Updated labeling in the illustrations throughout the text increases readability, which in turn enhances students' ability to comprehend key concepts. As a corollary to the book's updated art program, the design and color palette have also been modernized.

Proven Features

Visual Impact Ideal for visual learners, this book's state-of-the-art approach helps students visualize key processes and understand important topics.

Biochemical Connections The Biochemical Connections highlight special topics of particular interest to students. Topics frequently have clinical implications, such as cancer, AIDS, and nutrition. These essays help students make the connection between biochemistry and the real world.

Apply Your Knowledge The Apply Your Knowledge boxes are interspersed within chapters and are designed to provide students with problem-solving experience. The topics chosen are areas of study where students usually have the most difficulty. *Solutions* and *problem-solving strategies* are included, giving examples of the problem-solving approach for specific material.

Early Inclusion of Thermodynamics Select material on thermodynamics appears much earlier in the text. Chapter 1 includes sections on *Energy and Change, Spontaneity in Biochemical Reactions,* and *Life and Thermodynamics.* Also, Chapter 4 contains an extended section on *Protein Folding Dynamics.* We feel it is critical that students understand the driving force of biological processes and see that so much of biology (protein folding, protein–protein interactions, small molecule binding, etc.) is driven by the favorable disordering of water molecules.

Summaries and Questions Each chapter closes with a concise summary, a broad selection of questions, and an annotated online bibliography. As stated previously, the summaries have been completely revised to reflect the in-text "Q & A" framework. The number of questions has been expanded to provide additional self-testing of content mastery and more homework material. These exercises fall into four categories: *Recall, Reflect and Apply, Biochemical Connections,* and *Mathematical.* The *Recall* questions are designed for students to quickly assess their mastery of the material, while the *Reflect and Apply* questions are for students to work through more thought-provoking questions. *Biochemical Connections* questions test students on the *Biochemical Connections* essays in that chapter. The *Mathematical* questions complete the selection of exercises. These questions are quantitative in nature and focus on calculations.

Organization

Because biochemistry is a multidisciplinary science, the first task in presenting it to students of widely varying backgrounds is to put it in context. Chapters 1 and 2 provide the necessary background and connect biochemistry to the other sciences. Chapters 3 through 8 focus on the structure and dynamics of important cellular components. Molecular biology is covered in Chapters 9 through 14. The final part of the book is devoted to intermediary metabolism.

Some topics are discussed several times, such as the control of carbohydrate metabolism. Subsequent discussions make use of and build on information students have already learned. It is particularly useful to return to a topic after students have had time to assimilate and reflect on it.

The first two chapters of the book relate biochemistry to other fields of science. Chapter 1 deals with some of the less obvious relationships, such as the connections of biochemistry with physics, astronomy, and geology, mostly in the context of the origins of life. Functional groups on organic molecules are discussed from the point of view of their role in biochemistry. This chapter goes on to the more readily apparent linkage of biochemistry with biology, especially with respect to the distinction between prokaryotes and eukaryotes, as well as the role of organelles in eukaryotic cells. Chapter 2 builds on material familiar from general chemistry, such as buffers and the solvent properties of water, but emphasizes the biochemical point of view toward such material.

Chapters 3 through 8, covering the structure of cellular components, focus on the structure and dynamics of proteins and membranes in addition to giving an introduction to some aspects of molecular biology. Chapters 3, 4, 6, and 7 deal with amino acids, peptides, and the structure and action of proteins, including enzyme

catalysis. Chapter 4 includes more material on thermodynamics, such as hydrophobic interactions. Chapter 5 focuses on techniques for isolating and studying proteins. The discussion of enzymes is split into two chapters (Chapters 6 and 7) to give students more time to fully understand enzyme kinetics and enzyme mechanisms. Chapter 8 treats the structure of membranes and their lipid components.

Chapters 9 through 14 explore the topics of molecular biology. Chapter 9 introduces the structure of nucleic acids. In Chapter 10, the replication of DNA is discussed. Chapter 11 focuses on transcription and gene regulation. This material on the biosynthesis of nucleic acids is split into two chapters to give students ample time to appreciate the workings of these processes. Chapter 12 finishes the topic with translation of the genetic message and protein synthesis. Chapter 13 focuses on biotechnology techniques, and Chapter 14 deals with viruses, cancer, and immunology.

Chapters 15 through 24 explore intermediary metabolism. Chapter 15 opens the topic with chemical principles that provide some unifying themes. Thermodynamic concepts learned earlier in general chemistry and in Chapter 1 are applied specifically to biochemical topics such as coupled reactions. In addition, this chapter explicitly makes the connection between metabolism and electron transfer (oxidation–reduction) reactions.

Coenzymes are introduced in this chapter and are discussed in later chapters in the context of the reactions in which they play a role. Chapter 16 discusses carbohydrates. Chapter 17 begins the overview of the metabolic pathways by discussing glycolysis. Discussion of glycogen metabolism, gluconeogenesis, and the pentose phosphate pathway in Chapter 18 provides a base for treating control mechanisms in carbohydrate metabolism. Discussion of the citric acid cycle is followed by the electron transport chain and oxidative phosphorylation in Chapters 19 and 20. The catabolic and anabolic aspects of lipid metabolism are dealt with in Chapter 21. In Chapter 22, photosynthesis rounds out the discussion of carbohydrate metabolism. This chapter goes into the plant origin of antimalarials and has a connection to the Hot Topic of malaria. Chapter 23 completes the survey of the pathways by discussing the metabolism of nitrogen-containing compounds such as amino acids, porphyrins, and nucleobases. Chapter 24 is a summary chapter. It gives an integrated look at metabolism, including a treatment of hormones and second messengers. The overall look at metabolism includes a brief discussion of nutrition and a somewhat longer one of the immune system.

This text gives an overview of important topics of interest to biochemists and shows how the remarkable recent progress of biochemistry impinges on other sciences. The length is intended to provide instructors with a choice of favorite topics without being overwhelming for the limited amount of time available in one semester.

Alternative Teaching Options

The order in which individual chapters are covered can be changed to suit the needs of specific groups of students. Although we prefer an early discussion of thermodynamics, the portions of Chapters 1 and 4 that deal with thermodynamics can be covered at the beginning of Chapter 15, "The Importance of Energy Changes and Electron Transfer in Metabolism." All of the molecular biology chapters (Chapters 9 through 14) can precede metabolism or can follow it, depending on the instructor's choice. The order in which the material on molecular biology is treated can be varied according to the preference of the instructor.

Supporting Materials

Please visit http://www.cengage.com/chemistry/campbell/biochemistry8e for information about student and instructor resources for this text.

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A Final Note from Mary Campbell

I thank my family and friends, whose moral support has meant so much to me in the course of my work. When I started this project years ago, I did not realize that it would become a large part of my life. It has been a thoroughly satisfying one.

A Final Note from Shawn Farrell

I cannot adequately convey how impossible this project would have been without my wonderful family, who put up with a husband and father who became a hermit in the back office. My wife, Courtney, knows the challenge of living with me when I am working on four hours of sleep per night. It isn't pretty, and few would have been so understanding. I would also like to thank David Hall, book representative, for starting me down this path, and the late John Vondeling for giving me an opportunity to expand into other types of books and projects.

BIOCHEMISTRY

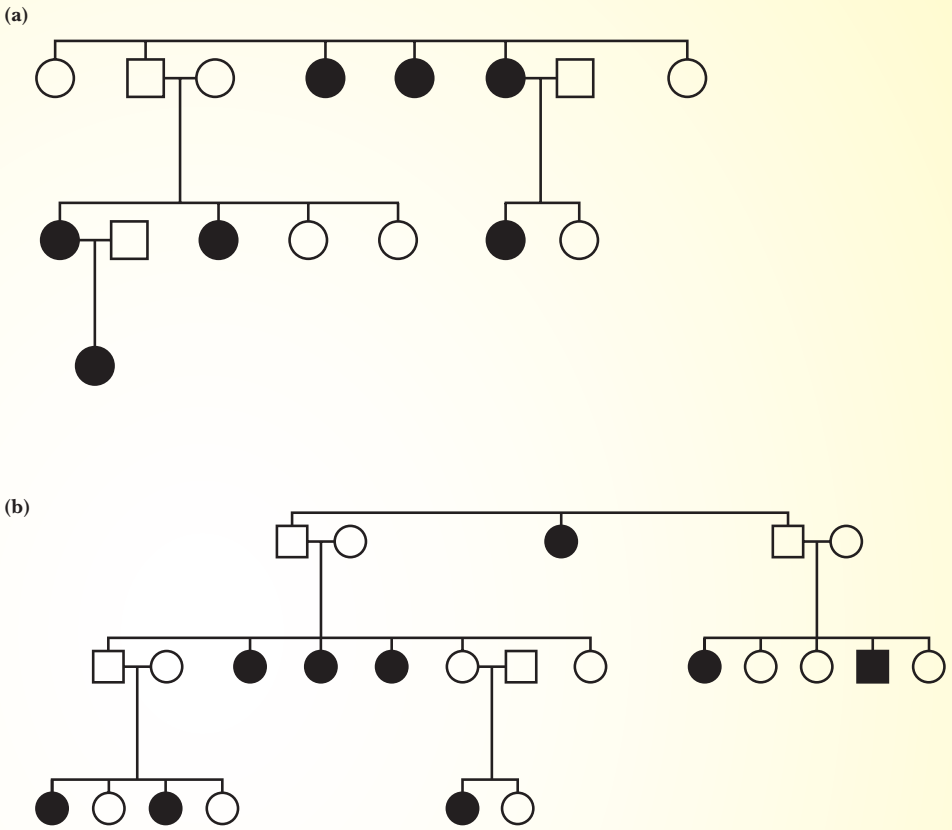
HOT TOPICS

The Genetics of Breast Cancer

Of all the ailments that harm us, cancer certainly qualifies as a dread disease. The level of fear is, if possible, even higher with breast cancer. It is no surprise that an enormous amount of research has been devoted to the topic. In October 2012, the Cancer Genome Atlas Network published results that represented the culmination of decades of work. They called their results "comprehensive molecular portraits of human breast tumors." Let us look at how this story developed.

One of the first major studies focused on the genetic aspects of breast cancer. In 1990, a group of researchers in California studied 23 extended families with 146 cases of breast cancer. They focused on the number of affected individuals in a given family, especially those with early onset of the disease. The research method combined classic genetic analysis with DNA studies. Statistical analysis of cancer incidence in the family trees indicated that these cancers were not sporadic cases that arise from somatic mutation in individuals, but were genetically linked. In two of these families, men developed breast cancer. It is very unusual for men to do so, but it does occur. DNA was obtained from blood samples taken from 329 members of the families being studied. Southern blots (see Chapter 13 for details of how this DNA hybridization technique works) gave information about the homology of DNA samples among various family members. The change in DNA linked to occurrence of breast cancer was localized to chromosome 17, characterized by a variable number of tandem repeats of specific DNA sequences. This result was the first milestone in determining the genetics of breast cancer. The gene is designated *BRCA1*.

At about the same time, researchers in Utah were carrying out similar investigations on the genetics of breast cancer.



Family trees showing the genetic distribution of breast cancer in two families. Each horizontal row represents a generation. Males are shown as squares and females as circles. A solid symbol represents an individual who developed breast cancer, and an open symbol represents nonoccurrence. Note that men can develop breast cancer.

They used genetic and cytologic analysis to study the relationship between proliferative breast disease (PBD) and breast cancer. PBD is a term used to describe several kinds of nonmalignant breast lesions. They found that a high incidence of PBD, which was detected cytologically, strongly correlated with high risk for breast cancer due to family history. PBD may represent a precancerous state. Their results led them to consider the possibility that development of breast cancer involves several genes.

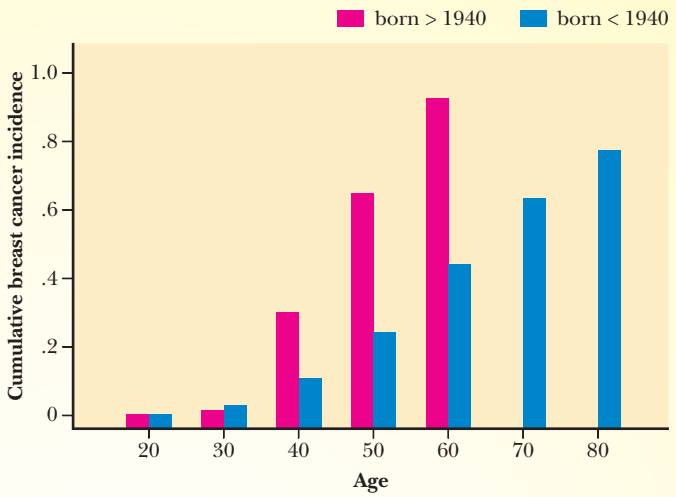
In 1994, an international group of researchers announced the discovery of a second gene for breast cancer susceptibility, one that was not linked to *BRCA1*. This second gene, designated *BRCA2*, is located on chromosome 13. Some significant differences between the two genes were readily apparent. Both confer a high degree of susceptibility to breast cancer, but they differ in the degree of risk of ovarian cancer. The presence of *BRCA1* substantially increases the risk of ovarian cancer, but *BRCA2* does not do so. In addition, male breast cancer does not appear to be linked to *BRCA1*, but there is a small risk for men who carry the *BRCA2* gene. For women who carry these genes,

the probability of breast cancer at some point in their life is over 80%. Some women who have this genetic predisposition have opted for double mastectomies as a preventive measure.

In May 2013, the use of double mastectomies as a preventive measure received wide publicity when the actress Angelina Jolie announced that she had undergone this surgery. DNA testing had shown that she carried the mutation in the *BRCA1* gene that drastically increases the probability of developing breast cancer. In her contribution to the Opinion Pages of the *New York Times*, entitled *My Medical Choice*, Jolie indicated that she was strongly motivated to see that her children did not lose their mother at an early age. She made her choice public in the hope that other women could benefit from her experience. This news did indeed lead to worldwide news coverage and discussion of all aspects of breast cancer. A number of women chose to share their experience of mastectomies, both for prevention and treatment of cancer. These stories show that management of breast cancer has changed markedly over the years, in large part because of advances in research.

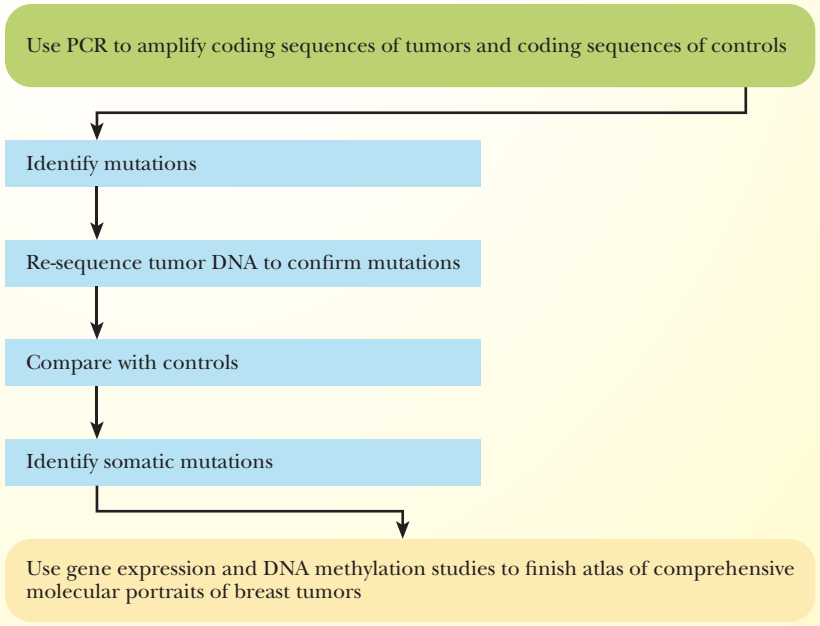
As time went on, it became apparent that the *BRCA1* and *BRCA2* genes are cancer suppressors, and that mutations in these genes in affected families increase the probability of cancer. A number of factors appear to affect the incidence of breast cancer. Some families have a low incidence of breast cancer until a case occurs with one of these mutations. In nearly all cases such as these, the mutation proved to be inherited from fathers. A number of other factors can and do affect the onset of cancer. For example, women with high levels of physical activity and normal weight in adolescence had a lower tendency to breast cancer than women who were sedentary or obese in adolescence. It also appears that risk is increasing with time. Women born before 1940 in affected families have high risk, but it is higher still for women born after 1940. Figure 1 shows a comparison of breast cancer incidence, particularly at early ages, for women born before and after 1940. Clearly, the risk is increasing.

Very soon afterward, the success of the Human Genome Project allowed access to the sequence of portions of the genome



■ **FIGURE 1 A comparison of breast cancer incidence for women with *BRCA1* and *BRCA2* mutations born before and after 1940, showing increasing risk with time.** (*Based on King, M., et al. (2003). Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2.* Science *302(5645), 645. Copyright © 2015 Cengage Learning®.*)

affected by these cancer-predisposing mutations. In 2006, extensively studied protein-encoding genes were sequenced using tissue samples from cancer patients. Breast tumors and colorectal tumors were used in the study because they are among the most common cancers. As Figure 2 shows, several rounds of polymerase chain reaction (PCR) amplification and subsequent sequencing were needed to accomplish this task. Sequences of the same genes in normal tissue were used as controls. Another part of the task was to select the somatic mutations in individuals that are characteristic of cancer and to distinguish them from germline mutations that are passed on to succeeding generations. An important result was the finding that each cancer included a number of mutations, rather than a single mutation responsible for cancer. The work done in this study uses methods down to, but not including, the portion shaded in yellow in Figure 2. We will see those techniques used in the development of the comprehensive atlas of mutations.



■ **FIGURE 2 Schematic diagram of the screening procedure used to identify cancer-associated mutations.** *(Adapted from Sjöblom, T., et al. (2006). The consensus coding sequences of human breast and colorectal cancers.* Science *314, 269.)*

In 2007, scientists building on the study done in the previous year were able to reach a number of conclusions about the genetic variations that lead to cancer. One striking result is that tumors contain a number of individual mutations. In a typical cancer, about 80 mutations occur, each of which causes an amino acid change. Statistical analyses suggest that not all of these mutations lead to cancer. The number of mutations that trigger or maintain cancer is probably fewer than 15. However, identifying the mutations is only a beginning. It does not give information about how the mutation leads to development of a tumor. Identifying the proteins encoded by the mutant genes is a step forward because it indicates the pathways that may be affected. Note the use of the plural pathways, not pathway. A few small changes in the efficiency of several reactions may well combine to be the triggering event in developing cancer. Insight into structural changes in proteins that catalyze reactions in these pathways can suggest therapeutic approaches. In this study, mutations were found in a number of enzymes, including the glycosylation enzyme *GALNT5*. Figure 3 shows the known crystal structure of this enzyme with bound substrate. The yellow spheres are the mutated amino acids, which lie close to the active site, superimposed on the structure. This information does not suggest any therapeutic advance, but it does suggest approaches to develop new therapies.

The work of the Cancer Genome Atlas Network built upon all this previous research, culminating in publication of a wide-ranging picture of the genetic changes in breast cancer (see the *Nature* article cited in the bibliography). Results from a number of methods went into the analysis. An analysis of anomalies in DNA methylation was one technique, and sequencing of exons was another. Gene expression analyses with both mRNA and miRNA also played a role. Gene expression by DNA was yet another method, as was analysis of single nucleotide polymorphisms (changes in one base in DNA). Even with automated techniques and the kinds of array processing described in Chapter 13, this work was an enormous undertaking. Refer to Figure 2 for an outline of the method. Note the techniques added to those used in earlier studies (the portion outlined in yellow).

These results have some correlation with clinical features, but the correspondence is not complete. We know, for example, that the chemotherapeutic agent herceptin is effective only in the kinds of cancer where the gene for Human Epidermal Growth Factor (HER2) is overexpressed. It is possible to determine whether this is the case in individual patients and to plan treatment accordingly. Ideally, the time will come when we can correlate all mutations with specific clinical features and plan treatment accordingly. That time is not here yet, but this kind of work brings it closer.

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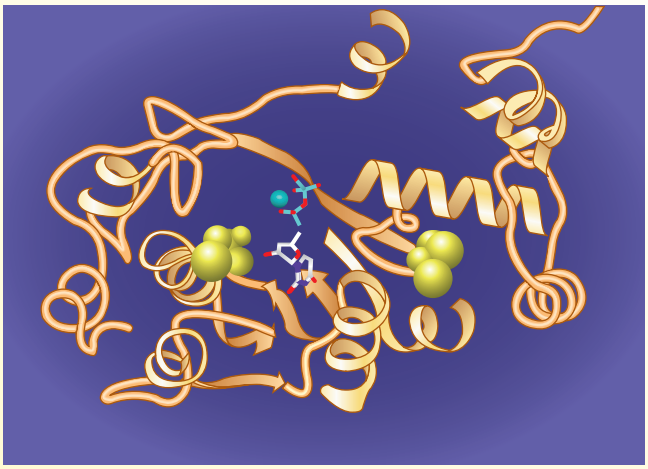


■ **FIGURE 3 Structure of the enzyme *GALNT5*. The yellow spheres are superimposed, showing amino acid mutations in a tumor.** *(Adapted from Macmillan Publishers Ltd: The Cancer Genome Altas Network, (2012). Comprehensive molecular portraits of human breast tumours.* Nature *490, 62.)*

Stem Cells: Science and Politics

Stem cells are the precursors of all other cell types. They are undifferentiated cells that have the ability to form any cell type as well as to replicate into more stem cells. Stem cells are often called progenitor cells because of their ability to differentiate into many cell types. A pluripotent stem cell is one that can give rise to all cell types in an embryo or in an adult. Some cells are called multipotent because they can differentiate into more than one cell type, but not into all cell types. The further from a zygote a cell is in the course of development, the less the potency of the cell type. The use of stem cells, especially embryonic stem (ES) cells, is an exciting field of research that really took off in the late 1990s.

History of Stem Cell Research

Stem cell research began in the 1970s with studies on teratocarcinoma cells, which are found in testicular cancers. These cells are bizarre blends of differentiated and undifferentiated cells. They were referred to as embryonal carcinoma (EC) cells. They were found to be pluripotent, which led to the idea of using them for therapy. However, such research was suspended because the cells had come from tumors, which made their use dangerous, and because they were aneuploid, which means they had the wrong number of chromosomes. As we shall see, the possibility of cells becoming cancerous is one of the major hurdles to overcome when we consider using stem cells for tissue therapy.

Early work with ES cells came from cells that were grown in culture after being taken from embryos. Researchers found that these stem cells could be grown in culture and maintained for long periods. Most differentiated cells, on the other hand, will not grow for extended periods in culture. Stem cells are maintained in culture by the addition of certain factors, such as leukemia inhibitory factor or feeder cells (nonmitotic cells such as fibroblasts). Once released from these controls, ES cells differentiate into all kinds of cells, as shown in Figure 1.

Stem Cells Offer Hope

Stem cells placed into a particular tissue, such as blood, differentiate and grow into blood cells. Others placed into brain tissue grow into brain cells. This is a very exciting discovery. Previously it had been believed that there was little hope for patients with spinal cord and other nerve damage, because nerve cells do not normally regenerate. In theory, neurons could be produced to treat neurodegenerative diseases, such as Alzheimer's disease or Parkinson's disease. Muscle cells could be produced to treat muscular dystrophies and heart disease. In one study, mouse stem cells were injected into a mouse heart that had undergone a myocardial infarction. The cells spread from an unaffected region into the infarcted zone and began to grow new heart tissue. Human pluripotent stem cells have been used to regenerate nerve tissue in rats with nerve injuries and have been shown to improve motor and cognitive ability in rats that suffered strokes. (See the articles by Sussman, by Aldhous, and by Donovan and Gearhart cited in the bibliography.) Results such as these led some scientists to claim that stem cell technology will be the most important advancement since cloning.

Truly pluripotent stem cells have been harvested primarily from embryonic tissue, and these cells show the greatest ability to differentiate into various tissues and to reproduce in cell culture. Stem cells have also been taken from adult tissues, because organisms always contain some stem cells even at the adult stage. These cells are usually multipotent, as they can form several different cell types, but they are not as versatile as ES cells. For this reason ES cells are the gold standard

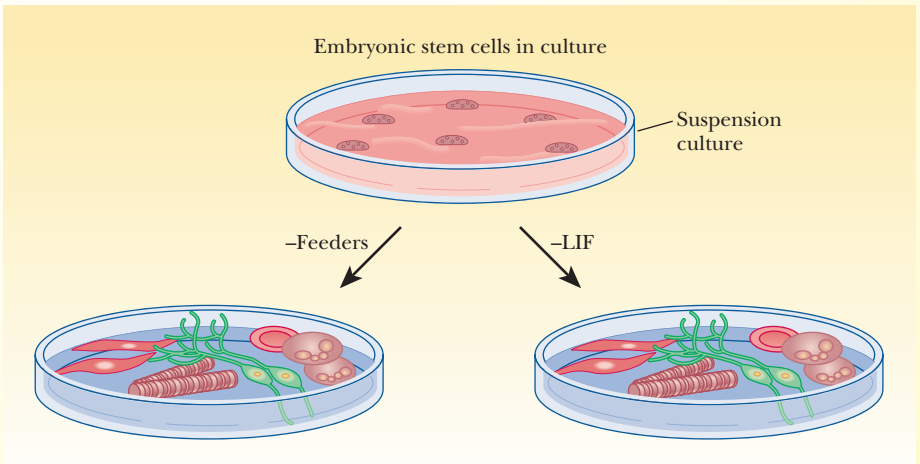
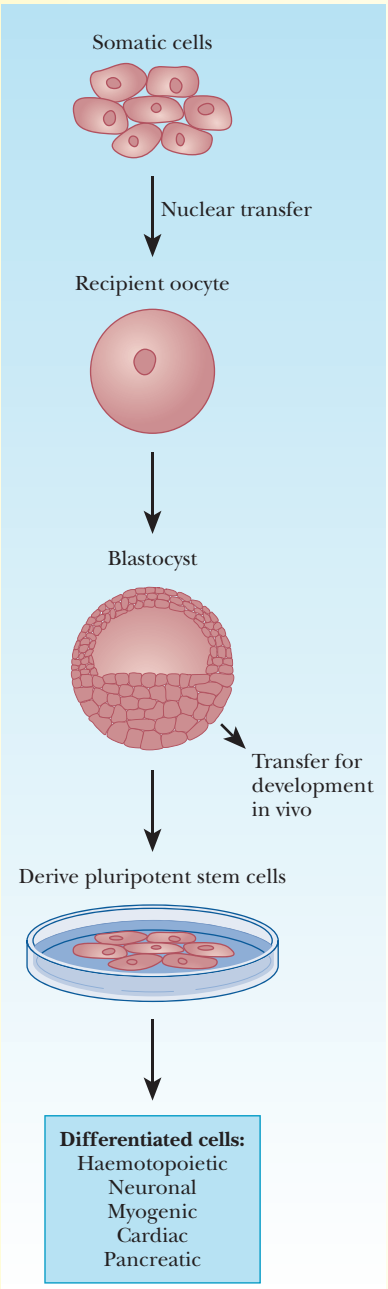


FIGURE 1 **Pluripotent embryonic stem cells can be grown in cell culture.** They can be maintained in an undifferentiated state by growing them on certain feeder cells, such as fibroblasts, or by using leukemia inhibitory factor (LIF). When removed from the feeder cells or when the LIF is removed, they begin to differentiate in a wide variety of tissue types, which could then be harvested and grown for tissue therapy. *(Taken from Donovan, P. J., and Gearhart, J. (2001). The end of the beginning for pluripotent stem cells.* Nature *414, 92–97.)*

for tissue therapy. The acquisition and use of stem cells can also be related to a technique called cell reprogramming, which is a necessary component of whole-mammal cloning, such as the cloning that produced the world's most famous sheep, Dolly. Most somatic cells in an organism contain the same genes, but the cells develop as different tissues with extremely different patterns of gene expression. A mechanism that alters expression of genes without changing the actual DNA sequence is called an epigenetic mechanism. An epigenetic state of the DNA in a cell is a heritable trait that allows a "molecular memory" to exist in the cells. In essence, a liver cell remembers where it came from and continues to divide and to remain a liver cell. These epigenetic states involve methylation of cytosine–guanine dinucleotides and interactions with proteins of chromatin. Mammalian genes have an additional level of epigenetic information called imprinting, which allows the DNA to retain a molecular memory of its germline origin. The paternal DNA is imprinted differently from the maternal DNA. In normal development, only DNA that came from both parents would be able to combine and to lead to a viable offspring.



■ **FIGURE 2 Reprogramming a somatic nucleus.** When transplanted into an oocyte, a somatic nucleus may respond to the cytoplasmic factors and be reprogrammed back to totipotency. These cytoplasmic factors erase the molecular memory of the somatic cells. Such cells can then be used to harvest pluripotent stem cells or to transfer a blastocyst into a carrier and develop an organism in vivo. *(Taken from Surani, M. A. (2001). Reprogramming of genome function through epigenetic inheritance.* Nature *414, 122–128.)*

Normally, the epigenetic states of somatic cells are locked in such a way that the differentiated tissues remain stable. The key to whole-organism cloning was the ability to erase the epigenetic state and to return to the state of a fertilized egg, which has the potential to produce all cell types. It has been shown that if the nucleus of a somatic cell is injected into a recipient oocyte (see Figure 2), the epigenetic state of the DNA can be reprogrammed, or at least partially reprogrammed. The molecular memory is erased, and the cell begins to behave like a true zygote. This can be used to derive pluripotent stem cells or to transfer a blastocyst into a mother-carrier for growth and development. In November 2001, the first cloned human blastocyst was created in this way, with the aim of growing enough cells to harvest pluripotent stem cells for research.

Science Takes on Politics

Controversy continues to rage worldwide over the use of ES cells. The issue is one of ethics and the definition of life. ES cells come from many sources, including aborted fetuses, umbilical cords, and embryos from in vitro fertilization clinics. The report about the cloned human embryonic cells added to the controversy. Under the Bush administration, in 2001 the U.S. government banned government funding for stem cell research, but it allowed research to continue on 21 existing embryonic cell lines. The questions driving the controversy include the following: Do a few cells created by therapeutic cloning of your own somatic cells constitute life? If these cells do constitute life, do they have the same rights as a human being conceived naturally? If it were possible, should someone be allowed to grow his or her own therapeutic clone into an adult?

Reuters/Shannon Stapleton

■ "As president, I will lift the current administration's ban on federal funding of research on embryonic stem cell lines created after August 9, 2001 through executive order."

In March 2009, President Obama announced that he was overturning the Bush administration's stem cell policy, giving new hope to stem cell researchers, although there was still plenty of red tape regarding which cell lines could be used and the ethics behind them. In December 2009, the National Institutes of Health (NIH) named the first 13 new lines approved for federal funding. Over a hundred new lines were expected to be approved by the end of 2010. In April 2009, the NIH released a draft of their

guidelines, which were seen as a huge improvement over the 21 legal cell lines approved before. Some restrictions are based on when the cell lines were derived and the ethics behind whether donors gave consent. The new cell lines had to be derived from surplus embryos donated by couples receiving fertility treatment. Stem cell lines derived from research cloning or somatic cell nuclear transfer were not eligible.

Despite the Obama executive order removing the Bush administration's restrictions on stem cell research, the field was dealt a serious blow in August of 2010. Two researchers, James Sherley and Theresa Deisher, sued the federal government on the grounds that it was illegal to fund research on ES cells and that such funding deprived them of their research into other cell types. Judge Royce Lamberth agreed and ruled that funding of human ES cell research had to be suspended. Government lawyers argued the other direction, claiming that the harm to the careers of the two scientists is outweighed by the harm to patients who are being deprived of potential cures and to taxpayers whose taxes were being wasted while the moratorium on research was in place. The Lamberth ruling threw stem cell research into a tailspin for two years.

Federal courts considered the lawsuit by Sherley and Deisher ultimately deciding to overturn the Lamberth ruling. Finally, in January of 2013, the supreme court decided not to hear the appeal of the federal courts ruling, essentially returning stem cell research to the position it held after Obama's executive order to allow federal funding of human ES cell research. While few believe the debate is over, at present stem cell researchers and patients are once again hopeful about their futures.

The Search for Less Controversial Stem Cells

Although the future of stem cell research is much brighter now than it was in 2010, researchers did not stand still during the difficult years. Rather, they began attempting to find ways to create stem cells that are not seen as controversial. Researchers at Columbia University proposed that embryos that had stopped dividing could provide a good source of stem cells. Fertility clinics that do in vitro fertilization have many more failed embryos than successful ones. At the point at which they realize the embryos are not viable and decide not to implant them, they would be able to harvest stem cells from them. Other attempts to generate stem cells involve using other tissues, such as cells from amniotic fluid. These have many of the properties of true stem cells, although they are not truly pluripotent.

Catherine Verfaillie of the University of Minnesota has purified cells from bone marrow called multipotent adult progenitor (MAP) cells. These cells are less versatile than embryonic stem cells, but her research suggested that they can be very useful for therapeutic techniques. In her research she used MAP cells to repopulate the blood cells of mice whose cells had been destroyed by radiation. (See the articles by Holden cited in the bibliography for more on methods for producing these less-controversial stem cells.) Several other labs began looking at other ways to produce cells with pluripotent properties without having to use embryonic tissue.

One of the biggest breakthroughs was made by Japanese researcher Shinya Yamanaka. While many other researchers were taking the new ES cells and working with controlling how to correctly differentiate them into target tissues, he took an opposite approach and began looking for ways of creating stem cells from regular somatic cells. He hypothesized that specific proteins would be found in embryonic cells but not in differentiated cells. He thought that if he could introduce the genes for these proteins into differentiated cells, he might be able to convert them back to a pluripotent state. After four years of research, he had uncovered 24 factors that would transform skin fibroblast cells into pluripotent cells in mice, and found that these cells were almost identical to stem cells. He then found that by introducing four specific genes into the fibroblast cells, he could accomplish the same thing. In 2006 he published his landmark article in which he identified these four critical genes as *Oct3/4, Sox2, c-Myc,* and *Klf4.* The cells derived from this process are called induced pluripotent stem cells (iPS cells). Yamanaka and other researchers have derived iPS cells from many tissues, including liver, stomach, and brain. These cells show some of the same abilities as true stem cells and have been turned into skin, muscle, cartilage, and nerve cells. In 2007, researchers in the United States extended the technique to create human iPS cells in work recognized as the first runner-up in *Science* magazine's *Breakthrough of the Year.*

There are two concerns with the original research that led to iPS cell production. First was the use of the gene *c-Myc*, which is a powerful cancer gene. In essence, making pluripotent cells can be looked at as being very similar to making cancer cells, and the two have many of the same properties. Further research showed that in mice, the *c-Myc* can be avoided and iPS cells can still be produced. The other risk is that the four genes were delivered using retroviruses, which, as we saw in Chapter 13, is a procedure that carries its own risks. This second danger was addressed in November 2008, when Matthias Stadtfeld and coworkers generated iPS cells without viral integration by using the common cold adenovirus. The adenovirus was able to produce the required cofactors in the cells without integrating into the host cells' DNA. Their iPS cells showed all of the ability of the iPS cells generated with the retroviruses. This is thought to be a safer alternative.

Scientists know more about how to create iPS cells than they do about why their techniques work, but they continue to search for the reasons. In 2009, Yamanaka, as well as several other researchers, simultaneously published evidence that a critical part of iPS production is the suppression of *p53*, a tumor suppressor gene discussed in Chapter 14. This leads to a tricky situation, however. To make the iPS cells, the *p53* must be suppressed, but for the cells to be stable and useful once created, this transcription factor must be reactivated. The creation of iPS cells was quite a breakthrough, but the new kid on the block has still not convinced many people. The most recent comparisons of iPS cells and traditional ES cells indicates that ES cells are easier to differentiate into target tissues than their iPS counterparts, as shown in Figure 3.

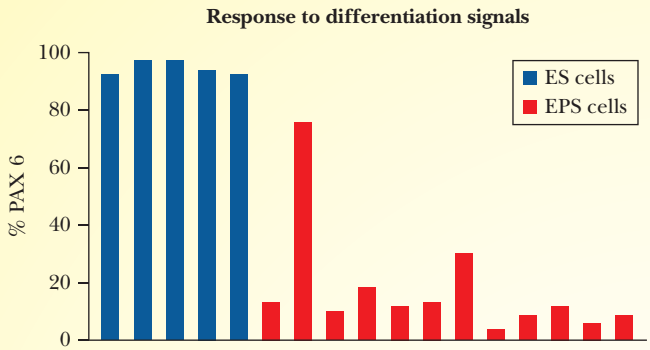


FIGURE 3 For more on the initial production of iPS cells, see the articles by Hornyak and by Stadtfeld in the bibliography. *(From Vogel, G. (2010). Reprogrammed cells come up short, for now.* Science *327 (5970), 1191. Reprinted with permission from AAAS.)*

Cutting Out the Middleman

Whether using ES cells or iPS cells, the original research that headed toward tissue therapy involved several changes to cell type. First a cell had to be turned into a pluripotent cell. Then the pluripotent cell had to be converted to the desired tissue type. The newest process involves avoiding this transition and changing one cell type directly into another one. In late 2008, researchers at Harvard University used cell reprogramming techniques to change one type of pancreatic cell in mice into the beta cells that produce insulin. This technique allows the direct programming of one cell type into another. After sifting through 1000 transcription factors, the researchers found that by turning on only three genes in the exocrine cells of the mouse pancreas, they could change exocrine cells to insulin-producing beta cells. A similar technique, called lineage switching, involves another way to change cell types, in this case by moving the cell back toward a less differentiated cell until a branch point and then redifferentiating it into something else. Such feats have obvious implications for the treatment of diabetes and many other diseases.

Many Paths to Reprogramming

Researchers interested in studying paths to cell reprogramming have a variety of paths to choose from these days, as shown in Figure 4. Path A shows nuclear transfer to an unfertilized egg, such as the process that led to the cloned sheep, Dolly. Path B shows how a differentiated cell from the stomach can be used to regress to an undifferentiated state in the form of an induced pluripotent cell. Path C shows a lineage switch where a macrophage becomes a lymphocyte. Path D shows the direct conversion of an exocrine cell to an endocrine beta cell.

Are Stem Cells the New Snake Oil?

No ethical researcher would tell you that we will be healing severed spinal cords in the next year or so, despite the many promising experiments done on animal models. Given the difficulty with repairing nerve tissue, the fact that any improvement can be seen is remarkable, but it will be many years or decades before treatments are readily accessible. There is still a lot to learn about all forms of cell reprogramming. For example, even though researchers were able to transform mouse pancreatic cells from one type to another, approval for human trials would depend on a better understanding of exactly why the process worked, something that is still a mystery.

Unfortunately, the hope offered by stem cells has spawned many clinics that promise results to desperate clients. A dozen or more companies produce stem cells for clinical use, and clinics exist in several countries, including Turkey, Azerbaijan, the Dominican Republic, the Netherlands, and China. Advertising campaigns from such companies have attracted many patients willing to spend more than $20,000 for stem cell therapy, but there is little proof yet that any of the claims of these clinics are valid. Many reputable scientists in the stem cell research world are currently investigating

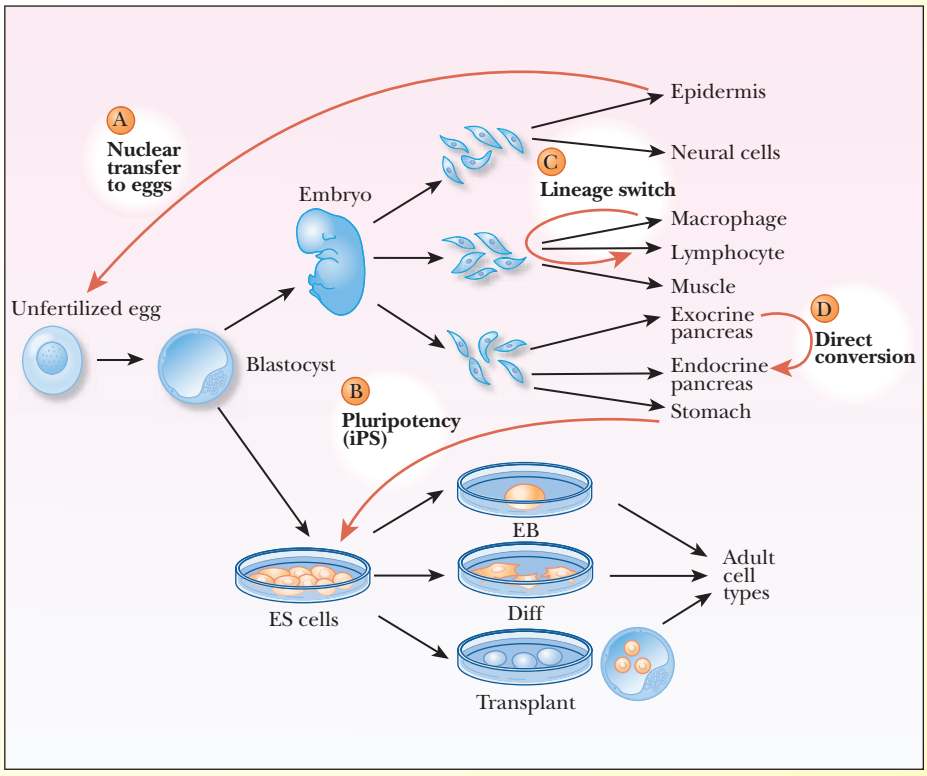


FIGURE 4 Many paths to reprogramming. See text for details. *(From Gurdon, J. B., and Melton, D. A. (2008). Nuclear reprogramming in cells.* Science *322 (5909), 1811. Reprinted with permission from AAAS.)*

some of the claims and facilities of these companies. (See the article by Enserink cited in the bibliography for a complete description of the state of human stem cell therapy clinics.) The political fight over stem cells continues in the United States. Even celebrities have come forward to endorse stem cell research and politicians that support it. Actor Michael J. Fox, himself a victim of Parkinson's disease, is a most vocal proponent for stem cell research (see Figure 5).

While the popular press has focused on the potential use of stem cells for tissue therapy to cure diseases, another very important use of stem cells is called disease modeling. Scientists can take cells from individual patients, create iPSC's and grow them in culture. These cells have the same genetic disease as the patient. With a suitably expanded pool of cells to work with, they can then study the effect of drugs on the patient's cells, essentially allowing direct experimentation on a virtual "patient in a petri dish." While not as flashy as the potential cure of a severed spinal cord via tissue therapy, in the short term disease modeling will allow uncontroversial use of a patient's own cells to help treat a disease (see the article by Vogel in the bibliography).

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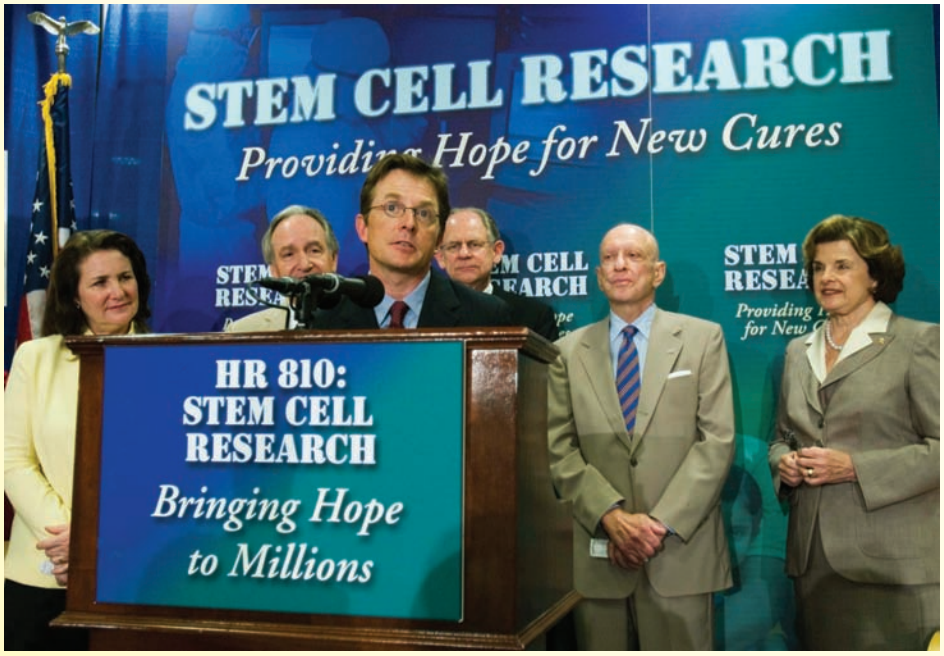


■ **FIGURE 5** **Celebrity activists.** Michael J. Fox has been interviewed many times regarding the U.S. policy on stem cell research. He has also campaigned for politicians who support it.

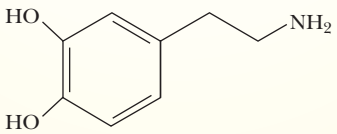
The Science of Happiness and Depression

Whether we think about it or not, happiness is an important issue in our lives. In the United States, we have always thought about the fundamental importance of happiness, which is why the founding fathers included "life, liberty, and the pursuit of happiness" in the Declaration of Independence. How much time we spend pondering the nature of happiness is a reflection on the nature of our lives and how hard we have to struggle to survive. A generation ago, people were too busy trying to put food on the table to worry about silly things like whether they were happy. In the 21st century, however, at least in first-world countries, people have more time and luxury to think about it.

The Brain and Pleasure

To begin to understand the nature of happiness, we must first be able to understand how the brain works with respect to the emotions that we associate with happiness. For decades scientists have been trying to determine where in the brain the "happiness centers" are and how they work. In the 1950s, a psychiatrist named Robert Heath did some of the pioneering work in mapping the brain by implanting electrodes into the brains of patients who suffered from a wide variety of afflictions, including epilepsy, schizophrenia, and depression. He was trying to find the biological seat of the disorders by stimulating regions in the brain, and he made some remarkable discoveries. Patients who were catatonic with despair would smile, talk, and laugh while areas of their brains were stimulated, but once the stimulation ended, they returned to their catatonic states. To continue the study, Heath fitted a handful of patients with electrodes where they could control their own stimulation. One patient self-stimulated 1500 times in a 3-hour session. These experiments helped to define structures in the brain that would be called the "pleasure center," and the theories from these experiments lasted for decades. In the years that followed, many researchers used animal models to demonstrate similar results. While it might not be intuitive that one can tell if a rat is depressed or not, there are certain behaviors that are common to all mammals, and researchers use these cues to determine whether nonhuman animals are depressed.

An underlying assumption in the early work, however, was that repetition implied that the individual was deriving pleasure from the response. What if this were not always the case? In the early 2000s, Morten Kringelbach and Kent Berridge (see the article, "The Joyful Mind," in the bibliography) began to question this underlying assumption. Many known addictive behaviors can be found where an individual repeats an action obsessively but does not seem to truly enjoy the object of his obsession. Using animal models, they began to study the "like versus want" conundrum. The regions of the brain identified in the 1950s are positioned at the front of the brain and are activated by the neurotransmitter dopamine (shown below), released by neurons that originate near the brainstem.



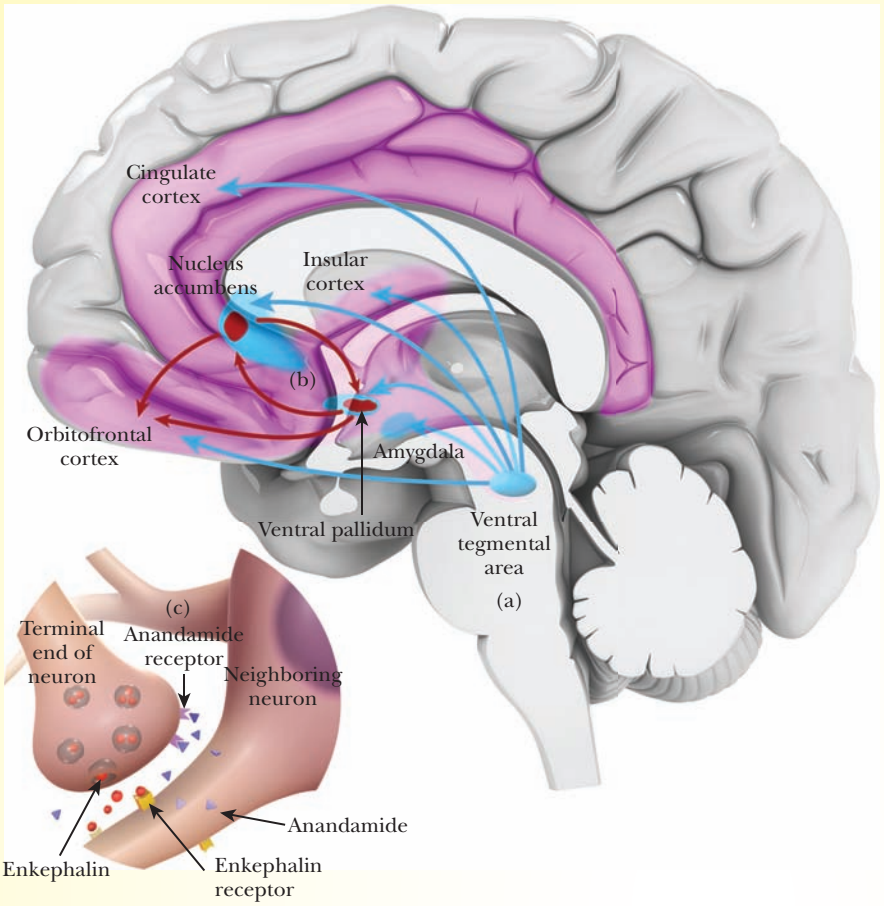
Dopamine

Kringelbach and Berrige hypothesized that if these areas of the brain were truly the pleasure centers, then flooding them with dopamine or removing dopamine receptors would alter an animal's response to a pleasurable stimulus, but that is not what they found. They used knockout mice (Chapter 13) that lacked a protein which would remove dopamine once it had been released. Thus, these animals would have more dopamine in their systems. If the dopamine was leading to pleasure, they should be "happier" mice when faced with pleasurable stimuli. However, what they found was that the mice would seek pleasurable stimuli, such as sweet foods, more actively than their normal counterparts, but once they attained the reward, they did not seem to enjoy it more. Rodents that are depleted of dopamine show no interest in sugary treats at all. They will starve to death unless actively nursed. Dopamine, therefore, does not seem to be the single neurotransmitter of pleasure, but is highly linked to motivation to attain pleasure, a subtle distinction. In human studies, dopamine also seems more related to a person's perception of how much they want something rather than how much they actually enjoy it. This was also confirmed by studies of people addicted to recreational drugs, many of which act by flooding the brain with dopamine. The addiction persists long after the pleasurable sensation associated with the drug subsides. Alcoholics and drug addicts often "have to" ingest their particular poison even if they don't want to.

The original work from the 1950s focused on an area of the brain near the brain stem, now called the ventral tegmental area, as shown in Figure 1.

This area communicates with several other areas of the brain as shown, but is now believed to be related to the wanting part of pleasure rather than the enjoying part. Of course, wanting is a part of pleasure as well. For example, receiving a gift you really wanted is often more rewarding than receiving one you did not.

Without discounting the importance of wanting, scientists looked to find other areas of the brain that were related to pleasure, and these have become known as hedonic hot spots. One of these is



■ **FIGURE 1 Wanting and Liking.** Evidence shows that there is a difference between wanting and liking, although they are related. In (a), several neural pathways associated with wanting begin in the ventral tegmental area and are propagated to other parts of the brain (blue arrows). These pathways of wanting are often associated with release of the neurotransmitter dopamine. The pathways were once thought to be the only pathways related to pleasure. More recently, researchers focused on other areas that are related to liking (b). These involve hedonic hotspots (shown in red), such as the nucleus accumbens and the ventral pallidum. The chemistry involved in these hedonic hotspots is shown in (c). A pleasurable stimulus, like the taste of chocolate, prompts the terminal end of a neuron to release enkephalins, which migrate to a neighboring neuron where they bind to enkephalin receptors. This releases another neurotransmitter, anandamide, which is the body's natural version of the active ingredient of marijuana, THC. The anandamide moves back to the anandamide receptors on the first neuron, setting up a feedback loop that extends the feeling of pleasure. *(AXS Biomedical Animation Studio, Inc.)*

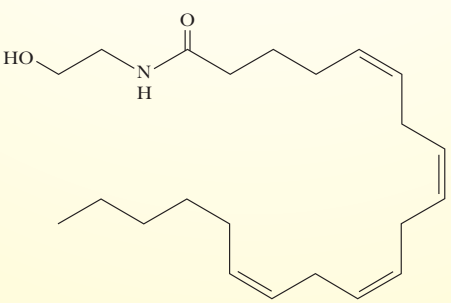
found in a subregion of the nucleus accumbens (shown in red in Figure 1). Another is found near the ventral palladium (also shown in red). Kringelbach and Berridge identified these by finding areas in the brains of rodents that, when stimulated, would increase the outward signs of enjoyment. The pathways that are related to feelings of pleasure in these structures are not based on dopamine. Rather they are based on peptide hormones discussed in Chapter 3 called enkaphalins (shown below), which are pentapeptides:

Tyr—Gly—Gly—Phe—Met

or

Tyr—Gly—Gly—Phe—Leu

Enkephalins bind to opioid receptors in nearby neurons. These neurons then release another hormone called anandamide (shown below):



Anandamide

Anandamide is the body's natural version of the THC molecule found in marijuana, and it is thought to regulate and propagate the feeling of pleasure initiated by the enkephalins.

While each of the hedonic hot spots is small, as shown in Figure 1, they make an interrelated complex with many parts of the brain. It is quite complicated and undoubtedly will be studied for years to come, but the separation of wanting and liking itself was a milestone for psychology.

Don't Worry—Be Happy

Some believe it is caused by our rat-race lifestyles. Others think it is due to toxins. Others think it is due to poor nutrition. Whatever the cause, there is no doubt that millions of Americans suffer from depression. The pharmaceutical industry and the fields of psychiatry and psychology are heavily funded by revenues from patients fighting depression. But before we talk about big pharma and antidepressants, let's look at some of the research on safer and cheaper options. In the last few years, a deficiency in many compounds has been found to correlate with depression.

Fatty Acids

In early 2011, researchers reported online in the journal *Nature Neuroscience* that deficiencies in omega-3 polyunsaturated fatty acids alter functioning of the endocannabinoid system, a group of lipids and their receptors that are involved in mood, pain sensations, and other processes. They noted that mice subjected to a diet low in omega-3 polyunsaturated fatty acids have lower omega-3 levels in the brain, and this is associated with an alteration in the functioning of the endocannabinoid system, specifically a deficit in the signaling of the CB1 cannabinoid receptor in the prefrontal cortex of the brain. The cannabinoid receptors are a class of cell membrane receptors under the G-Protein–Coupled Receptor superfamily. Cannabinoid receptors are activated by three major groups of ligands endocannabinoids (produced by the mammalian body), plant cannabinoids (such as THC, produced by the cannabis plant), and synthetic cannabinoids. All of the endocannabinoids and plant cannabinoids are lipophilic, that is, fat-soluble, compounds.

The CB1 cannabinoid receptor has been linked to depressive disorders.

Studies followed over 600 cases of depression and showed that patients who had diets higher in trans fatty acids were almost 50% more likely to be depressed. While much research remains to be done, this could indicate that eating salmon and other sources of omega-3 fatty acids, as well as diets low in trans fatty acids, can help one's head as well as one's heart.

Vitamin D

A clinical trial is testing the effect of vitamin D supplementation on insulin resistance and mood in diabetic women. Increased insulin resistance (type 2 diabetes) has been associated with depression. Higher vitamin D levels have been associated with a reduced risk of depression, diabetes, and other ailments. The study is looking to administer 50,000 international units of vitamin D per week for 6 months to 80 stable type 2 diabetic women aged 18–70 with signs of depression. Participants will be evaluated at three time points for serum vitamin D levels and other factors. There is evidence to suggest that vitamin D supplementation may decrease insulin resistance. Type 2 diabetes is on the rise in America and some evidence exists that several factors, including diet and vitamin deficiencies, can lead to this disease, as well as the associated depression.

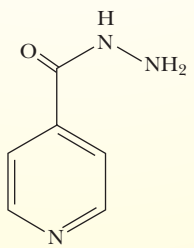
Vitamin B12

Vitamin B12 has the largest and most complex chemical structure of all the vitamins. It is unique among vitamins in that it contains a metal ion, cobalt. Cobalamin is the term used to refer to compounds having vitamin B12 activity. Methylcobalamin and 5-deoxyadenosyl cobalamin are the forms of vitamin B12 used in the human body. Most supplements contain cyanocobalamin, which is readily converted to 5-deoxyadenosyl and methylcobalamin. In mammals, cobalamin is a cofactor for only two enzymes, methionine synthase and L-methylmalonyl-CoA mutase.

Methylcobalamin is required for the function of the folate-dependent enzyme, methionine synthase. This enzyme is required for the synthesis of the amino acid, methionine, from homocysteine. Methionine in turn is required for the synthesis of S-adenosylmethionine, a methyl group donor used in many biological methylation reactions, including the methylation of a number of sites within DNA and RNA. Vitamin B12 deficiency is also believed to be involved in depression. Studies have found that up to 30% of patients hospitalized for depression are deficient in vitamin B12. A study of several hundred physically disabled women over the age of 65 found that women deficient in vitamin B12 were more likely to be severely depressed than non-deficient women. A study of over 3000 elderly men and women showed that those with vitamin B12 deficiency were almost 70% more likely to experience depression than those with normal vitamin B12 levels. The relationship between vitamin B12 deficiency and depression is not clear but may involve S-adenosylmethionine (SAMe). Vitamin B12 and folate are required for the synthesis of SAMe, a methyl group donor essential for the metabolism of several neurotransmitters.

Antidepressants—Bringing out the Big Guns?

People have been trying to cure depression for centuries using whatever their era's version of a "happiness pill" is. Until the 1950s, opioid drugs were used for depression, followed by amphetamines through the 1960s. In 1952, psychiatrist Max Luri was the first to use a dedicated antidepressant, Isoniazid, although it had previously been used to fight tuberculosis.



Isoniazid

In the decades that followed, there was an explosion in the number and types of drugs marketed to fight this poorly understood disease. A few of the major classes of antidepressant and how they function are shown below. Most of them inhibit the reuptake of the neurotransmitters that are associated with happiness and depression.

Selective serotonin reuptake inhibitors (SSRIs) are currently one of the most popular antidepressants. They block the reabsorption of the neurotransmitter, serotonin. Common examples of these are Prozac™, Zoloft™, and Lexapro™.

Norepinephrine reuptake inhibitors (NRIs) block the reuptake of this important neurotransmitter.

Serotonin-norepinephrine reuptake inhibitors (SNRIs) block both serotonin and norepinephrine reuptake, and represent another modern important class of antidepressant.

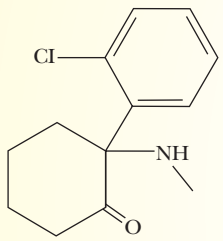
Norepinephrine-dopamine reuptake inhibitors (NDRIs) include Wellbutrin™.

Monoamine oxidase inhibitors (MAOIs) were an early class of antidepressant. They act by inhibiting the enzyme monoamine oxidase, which breaks down dopamine, serotonin, and norepinephrine. Due to complications with these drugs, they are not prescribed as often anymore.

There are several other classes of antidepressant, and each of the classes described have many examples of drugs in their class. Therefore, one might wonder why depression has not been eliminated altogether. Unfortunately, none of the drugs works as we would expect in a perfect world. Robin Marantz Henig describes in her article titled, "Lifting the Black Cloud" (see article in bibliography), an all-too-common case of a patient who spent a year on Paxil™ (an SSRI), but it destroyed her sex drive. She then tried Xanax™, an anti-anxiety drug. This brought back her sex drive but had other side effects. Then she tried Paxil again, followed by Lexapro (another SSRI), and then Pristiq™ (an SNRI). Then she went on Zoloft and Wellbutrin, the latter of which was supposed to offset the side effects of the Zoloft. Unfortunately, this trial-and-error approach is common with the prescription of antidepressants. They do not work for everyone and they don't work the same in everyone they affect. The most common

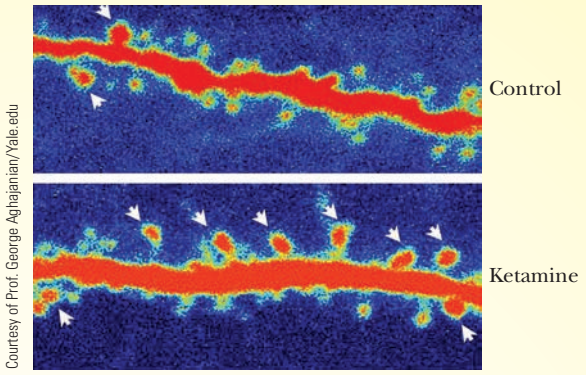
and popular since the 1980s and 1990s are the SSRIs and SNRIs, but they fail to work in 30% of cases. They also take a long time to start working, on the order of weeks. During those weeks, the chances of suicide are up to five times higher. Drug companies continue to look for better medicines, but the number of companies willing to make the effort is declining, which is bad news for the 15 million people in America alone who are clinically depressed.

Scientists are looking for an antidepressant that works faster. That would eliminate the dangerous waiting period as well as just make it more efficient to determine which one is right for a given individual. One way was to start with animal models using drugs that are known to be very fast. One such drug is ketamine.



Ketamine

Ketamine is an analgesic and a street drug called special K. In large doses it causes hallucinations, and in rodents it can be toxic to nerve cells, making it less than an ideal antidepressant for humans. However, it has shown to work very quickly and does almost immediately alleviate symptoms. Studies in rats have shown that the drug causes rats to start forming proteins needed to build new synapses between neurons in the prefrontal cortex, an area of the brain that behaves abnormally in depressed animals. Within 24 hours, the rats began to show new synaptic spines along the dendrites, which are projections that receive impulses from other neurons. In several studies, the more spines, the quicker the nerve impulses are transmitted and the less the animal shows signs of depression. In depression there is atrophy in the prefrontal cortex and the hippocampus. It is clear that the creation of the synaptic spines is one way that ketamine or other similar drugs help combat depression.



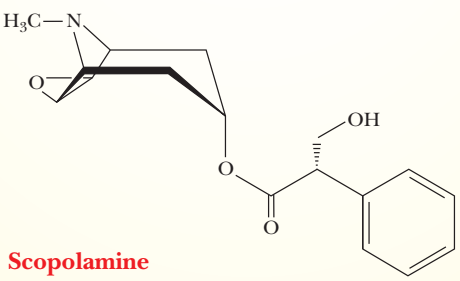
Courtesy of Prof. George Aghajanian/Yale.edu

■ **FIGURE 2** **In the presence of ketamine, neuronal dendrites are shown to sprout more spines than controls do.**

Figure 2 shows how dendrites sprout more spines when ketamine is administered.

Further research on ketamine has demonstrated that the neuronal growth is mediated through activation of an enzyme called mTOR, the same enzyme seen in the Hot Topic on aging. As ketamine is thought by many to be too risky for an antidepressant, scientists are looking for other drugs that activate mTOR. It is known that ketamine stimulates mTOR via preventing glutamate, a principal brain neurotransmitter from docking to its receptor, called an NMDA (N-methyl-D-aspartate) receptor, so finding drugs that block that site is an active area of research.

Another drug that acts quickly and which was already in use for another purpose—motion sickness—is scopolamine.



Scopolamine

Scopolamine influences a different system. It blocks the binding of the neurotransmitter acetylcholine to a type of receptor called a muscarinic receptor. In studies with over 40 subjects, intravenous injections of scopolamine were shown to relieve symptoms of depression within 3 days, with some patients reporting feeling better the next day. However, one stumbling block is the need for intravenous injections. This makes it not as practical as a pill or a skin patch, but these two techniques have not proved nearly as useful.

Besides the problem of how slowly many antidepressants work, researchers are also trying to tackle the issue that the known drugs do not work for all people. Some companies are targeting other receptor systems, such as the nicotinic receptors, so named because they respond to nicotine as well as their natural substrate, acetylcholine. However, some of the most exciting work involves different approaches instead of just coming up with a drug that will bind a receptor. One is methods to increase neurogenesis in the hippocampus, since it was shown that one of the issues seen with depressed patients was atrophy of the neurons in the brain. It had also been known that the SSRIs and SNRIs already in use act not only by affecting serotonin levels but also by increasing neuronal growth. The company Neuralstem is doing phase 1 trials of a pill form of a drug called NSI-189, which has shown promise in growing neurons in the hippocampus. Neuronal atrophy takes years to occur, and this pill will not be fast acting, but the company hopes that its effects will be long lasting.

Coming full circle, the most unique studies are once again focusing on serotonin, but with a completely different approach. They aim to increase serotonin receptors using gene therapy (Chapter 13). The gene being looked at is *p11*, which codes for a protein instrumental in the movement of serotonin receptors to the cell surface. Paul Greengard and colleagues at the Rockefeller Institute showed that in both

rodents and humans, individuals with depression-like behaviors also showed low levels of *p11*. Using an adenovirus carrier, Greengard put *p11* directly into the nucleus accumbens of deficient mice and their depressed behavior diminished. Currently studies are underway to see if a similar approach will work on monkeys. If it does, then scientists believe that gene therapy for humans will be a possibility.

There is lots of good news and bad news with respect to happiness in modern society. The good news is that most people have a quality of life that allows them to focus on the finer things, such as enjoyment and happiness. The bad news is that so many people struggle to find it, which is why so much research goes into finding a way to help people feel happier, or at least less depressed. This article just scratches the surface, but it is clear that the brain is a very complicated organ, as is the chemistry associated with it. It is almost ironic that a branch of science that is looking for ways to extend life span stumbled on an enzyme, mTOR, the inhibition of which correlates with longer lives, while another branch looking to cure depression found that activating the same enzyme correlates with reducing depression. We can only hope that we are not really left with a choice to make of either long life or happiness.

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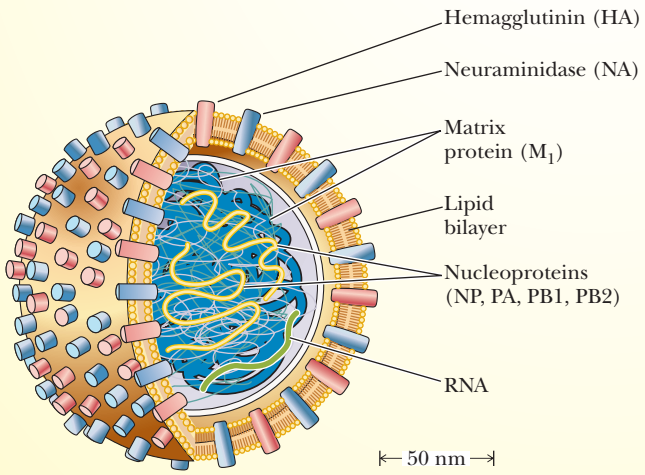
Humans versus Flu

In fall 2009, a common phrase heard in the schoolyard was, "he's got the swine," referring to the outbreak of swine flu which began in the spring of that year. Certainly anyone reading this book has had influenza—the flu—a disease that most people take for granted as an annoying fact of life, sometimes annually. There are frequent epidemics around the world, with some being very serious. In 1918, there was a worldwide flu pandemic that led to the deaths of 50 million people, one of the worst epidemics in history, surpassing even the black plague of the Middle Ages. By comparison, there are only about 40 million people today living with the HIV virus, and it has taken 30 years to get to that point. The flu virus has been with us for thousands of years and has never been fully controlled by modern medicine.

What Is a Flu Virus?

A single particle of the influenza virus (a virion) is a single-stranded RNA template strand genome with a protein coat that protrudes through a lipid bilayer envelope. Figure 1 shows the structural features of the influenza virus.

There are three major types, designated A, B, and C, depending on differences in the proteins. Influenza viruses cause infections of the upper respiratory tract that lead to fever, muscle pain, headaches, nasal congestion, sore throat, and coughing. One of the biggest problems is that people who catch the flu often get secondary infections, including pneumonia, which is what makes the flu potentially lethal. We are going to talk about the influenza A virus because, of the three, it is responsible for most human illness. The most prominent features of the virus envelope are two spike proteins. One is called hemagglutinin (HA), which gets its name because it causes erythrocytes to clump together. The second is neuraminidase (NA), an enzyme that catalyzes the hydrolysis of a linkage of sialic acid to galactose or galactosamine (see Chapter 16). HA is believed to help the virus in recognizing target cells. NA is believed to help the virus get through mucous membranes and enter cells. Sixteen subtypes of HA are known (designated H1–H16), and nine subtypes of neuraminidase (designated N1–N9) have been catalogued. H1, H2, H3, N1, and N2 occur in most of the known viruses that affect humans. Individual influenza A viruses are named by giving the subtypes of HA and NA—for example, H1N1 or H3N2. The virus that causes the avian influenza that has been in the news for several years is H5N1. The presence of the H5 protein affects humans, but so far to a lesser extent than the other HA subtypes. It does, of course, affect birds, with many fatalities among chickens, ducks, and geese. When the avian flu first broke out, it decimated flocks of domestic fowl around the globe.



■ **FIGURE 1 A cutaway diagram of the influenza virion.** The HA and NA spikes are embedded in a lipid bilayer that forms the virion's outer envelope. A matrix protein, M1, coats the inside of this membrane. The virion core contains the eight single-stranded segments that constitute its genome in a complex with the proteins NP, PA, PB1, and PB2 to form helical structures called neocapsids. (*Reprinted with permission from the Estate of Bunji Tagawa.*)

How Do the Various Flu Viruses Affect Humans?

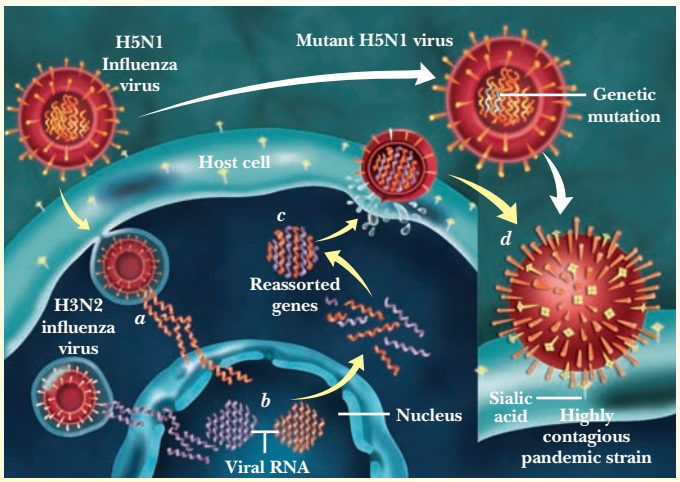
The nature of the virus subtype determines its effect on humans. The relevant factors to epidemiologists are transmissibility and mortality. For example, there have been only a few hundred cases of people contracting the avian flu worldwide, so its transmissibility is relatively low. However, of those known to have gotten the virus, more than 60% have died, so it is still a big concern. In contrast, the 2009 swine flu was the H1N1 variety, and it is more transmissible, leading to the first flu pandemic in 40 years, but it is far less deadly to those who get it. In many cases its symptoms are no worse than those of any common flu, and there have been few fatalities. One of the author's sons goes to a small school that had 32 cases of swine flu in a single day, only two less than would have been necessary to close the school for a week. While not feeling particularly well one day, they were sent to school anyway, a fact said author will not be allowed to forget, as it cost them a week off of school. One of them was later diagnosed with the swine flu.

Why Can't We Wipe Out the Flu?

While the flu has been with us for millennia, it is always changing, and it is the possibility of such changes that worries agencies responsible for public health, such as the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO). Mutations occur frequently with viruses, making it difficult to mount a good defense. One of the biggest worries is that a strain with a high mortality rate could mutate into one that is also very transmissible. Figure 2 shows how the deadly avian strain could potentially change.

In one possibility, the virus (the H5N1 avian virus in this example) mutates and changes its surface proteins, making it more able to bind to human cells and infect them (white path). The other possibility is that two viruses might infect the same cell (H5N1 and H3N2 in this example, yellow path). The viral RNAs could get mixed and produce reassorted genes, leading to different capabilities in a mutated new strain. Reassorted genes can happen anytime a host is infected by multiple strains of a virus. This can happen among humans, birds, and pigs, for example, considering that all three types of animals are frequently together, such as on a farm that has chickens and pigs.

The deadly flu of 1918 was also an H1N1 swine flu. Clues that the 2009 flu was not completely new came from the fact that young people were hit much harder than old people, whereas old people are usually targets for new flu viruses. This indicated that people who had been alive for many decades must have had some immunity to the 2009 swine flu. This clue helped lead to a quicker identification of the flu type. While separated by almost 100 years, the 1918 flu and the 2009 swine flu were so similar that mice given a vaccine against the 1918 virus made antibodies that completely neutralized the 2009 version. In 1997, a flu that was mostly human in origin was found in North American pigs. A year later researchers found another version that combined genes from human, avian, and swine sources, a triple reassortant. The 2009 swine flu was also a triple reassortant, which combined pieces from three different sources. Such combinations demonstrate that flu viruses do not stay contained in a single species for long. This is the main reason that scientists worry about what the next jumbling of flu genes will do. It is also why the CDC and WHO take every case of flu seriously. A combination that had the mortality of the avian flu with the transmissibility of the 2009 swine flu could lead to the next plague. Fortunately, it has not happened as of this writing.



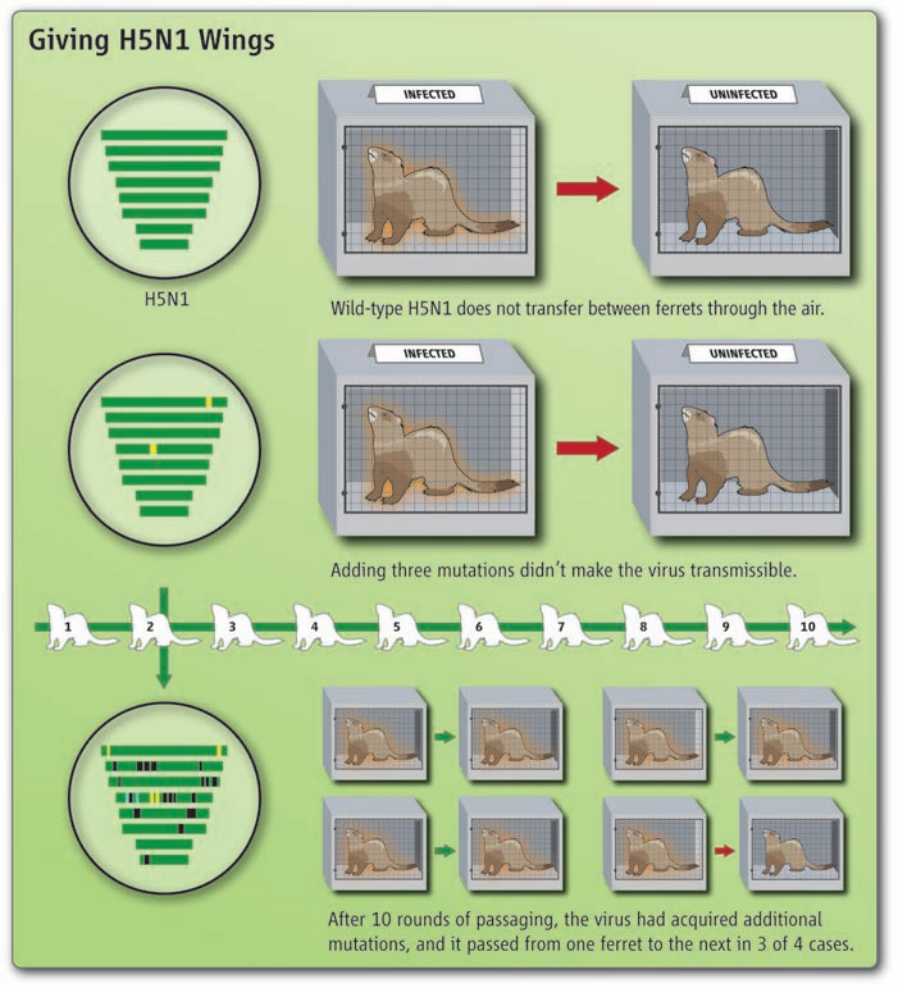
■ **FIGURE 2 Two possible strategies for mutating viruses.** The H5N1 strain might undergo a mutation that would make it bind more easily to the cell and therefore be more infective (white path). The H5N1 and H3H2 strains might both bind to the same cell and then mix their RNA to form reassorted genes (yellow path). *(Alice. Chen, 2005 for* Scientific American*)*

Too Much Information?

In the last decade, the world watched as three famous flu outbreaks made the news: SARS, avian flu, and swine flu. Researchers learned much about public health and epidemic preparedness from all of these. Most agreed that we were lucky that the highly transmissible swine flu was not very lethal. They also agreed that we were lucky that the deadly avian flu (H5N1) was not very transmissible. In fact, it did not seem to be passed from human to human very well, and certainly not through any airborne mechanisms. The big question was, therefore, what if H5N1 becomes airborne? The results could be catastrophic as humans have little to no immunity against the H5 variety.

Many researchers study disease transmissibility by using ferrets, a reasonable human model, and some of this research caught the eye of government agencies. In 2012 a debate raged in the scientific and government communities regarding how much information should be made public about studies from two different laboratories that worked on the "what if" question of mutating flu viruses. The work was done in the lab of Ron Fouchier, a virologist in Holland, and Yoshihiro Kawaoka, a virologist working in both Madison, Wisconsin and the University of Tokyo. The Fouchier study used caged ferrets in an attempt to show the airborne transmissibility of the native H5N1 virus, as shown in Figure 3.

The native H5N1 virus does not pass from ferret to ferret through the air (top row). The researchers made three mutations in the H5N1 virus, but these also did not pass from ferret to ferret (middle row). However, repeated passage of the virus from one ferret to the next led to further mutations. After 10 passages, the mutated virus was shown to be transmissible through the air (bottom row). This demonstrated that it was not terribly difficult for the virus to mutate to an airborne form. The conclusions from the Kawaoka studies were similar. However, for many months, these results were thought of as the most famous papers that were never published. In 2011, the U.S. National Science Advisory Board for Biosecurity (NSABB) recommended that



■ **FIGURE 3** **H5N1 can become airborne.** Native H5N1 does not pass from ferret to ferret through the air (top). After researchers made three mutations, the disease still did not pass through the air (center). After the virus was allowed to pass from ferret to ferret via contact, enough changes occurred that the virus did become airborne (bottom). *(From Enserink, M. (2012). Public at last, H5N1 study offers insight into virus's possible path to pandemic.* Science *336(6088), 1494–1497. Reprinted with permission from AAAS.)*

the results of these two papers not be published in full. The board felt that the full methodology would give would-be terrorists the information they needed to accomplish exactly what everyone feared. However, after an expert panel from WHO disagreed with the recommendations, the NSABB reviewed a modified version of the papers and changed its position, allowing publication of the papers.

Flu Vaccines

Besides watching the annual flu seasons and hoping that new deadly strains will not develop, government agencies also worry about having enough of the vaccines necessary to protect people against the flu. Many people get annual flu shots, and there is a reservoir of standard flu vaccines. Unfortunately, there is not nearly enough vaccine to cover everyone, especially if a new, more virulent strain comes about. The 2009 swine flu was another reminder of this problem because the spread of the disease had peaked long before enough vaccine could be made. One of the reasons is that few companies are willing to make vaccines anymore due to fear of litigation. In the mid-1970s there were 40 or 50 companies worldwide making flu vaccines, but now there are just a few. In 1976 the government prepared for a pandemic for a new strain of flu and suggested a massive vaccine program. While there was an epidemic, a pandemic never materialized, and there were people who sued the companies over side effects of the vaccines. Nowadays, governments commission the production of flu vaccines and take the litigation risk upon themselves.

While much of the response to the 1976 epidemic was seen as a disaster, at the same time what was learned about the virus did help lead to more rapid responses to modern flu strains, such as the avian flu and the 2009 swine flu. As with any virus that mutates quickly, making vaccines is always a challenging game of cat and mouse. Every year slightly different versions of the flu are found and labs attempt to match the vaccine to their best guess of what the "flu du jour" will be. Many people swear by their annual flu shot. Others would not think of getting one. Because many of the symptoms of the flu that we all experience are partially due to our own body's response to the vaccine, people may think they contracted the flu from the vaccine, although what they really experienced was just their own immunity in action.

Monoclonal Antibodies Take on the Flu

Besides generating vaccines against the flu virus, scientists are also trying to generate monoclonal antibodies that will fight the disease. Monoclonal antibodies are expensive to make, and in many cases the attempt is frustrated by the quickly changing nature of the virus. Therefore, researchers have been looking for an antibody that will attack part of the virus that does not change. In early 2009 two independent teams reported that they had created antibodies that would react with a portion of the flu virus's hemagglutinin (HA) protein. The good news was that the part of the hemagglutinin protein bound by the antibodies is relatively constant and does not change between strains.

The teams identified 10 different antibodies that recognized the H5 subtype found in the avian flu, and found they would also block 8 of the 15 other HA types. They tested their antibodies in mice both before and after they were dosed with lethal quantities of avian flu. Most of the rodents survived, indicating that these antibodies would work as prevention or as cure.

The medical community is excited about the prospects of adding another weapon to the eternal fight against the flu. Such antibodies can be used to provide an immediate passive immunity to people who do not respond well to vaccines, such as the elderly or those whose immune systems are compromised. They will also allow a strong countermeasure to an impending pandemic. The usual downside to such new discoveries is the cost. There are not enough cheap vaccines to meet the needs around the world, and many economically deprived countries have trouble getting them, especially an expensive monoclonal antibody. Still, many feel governments that can afford them would be wise to stockpile some of these new antibodies as protection against the next pandemic.

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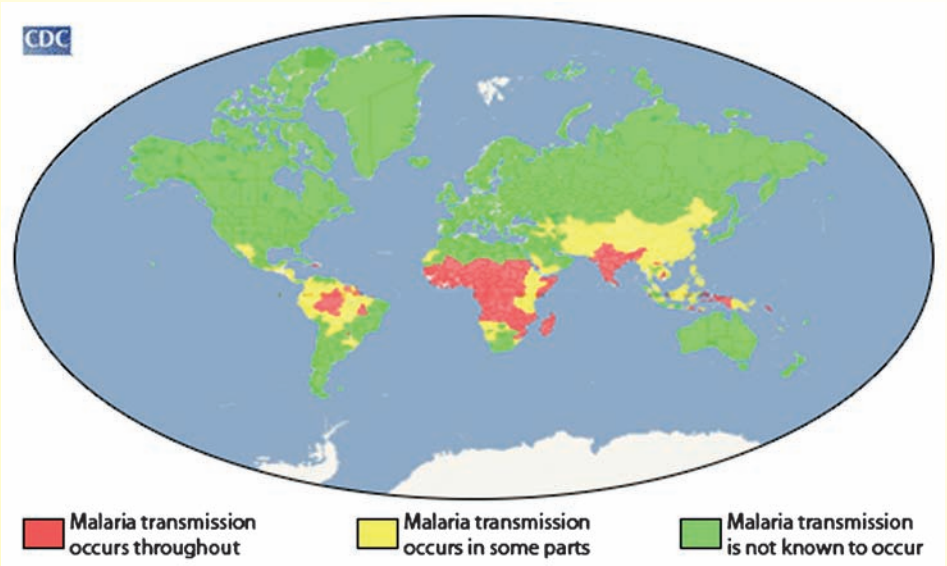
Malaria

Anyone who lives in a tropical climate is aware of malaria and the problems this disease can cause. Hundreds of millions of people are affected around the world every year, and approximately 1 million people die of it. It is no surprise that the topic is of great interest, especially in developing countries. A possible approach to the situation is to learn the basic facts about malaria, with a view to improving both prevention and treatment. The Centers for Disease Control and Prevention (CDC) maintain a website about malaria (www.cdc.gov/MALARIA), with links to specific information about various aspects of the disease. An especially important feature is an interactive map with updates on conditions in countries where malaria is endemic (Figure 1).

One of the useful pieces of information for anyone living in affected areas, as well as for visitors to those areas, is the specific parasite of the malaria-causing genus *Plasmodium* (a protist) that is prevalent there. *Plasmodium falciparum* is the species that causes the most severe infections, and most malaria research has focused on this parasite. The *Plasmodium* parasite attacks the liver of infected humans. The infection arises from the bite of a female *Anopheles* mosquito, the usual carrier, with transmission of the parasite from the saliva of the mosquito into the bloodstream of the human. The blood carries the parasite to the liver, where it reproduces. The parasite is in a haploid stage of its life cycle, called a sporozite, so it reproduces asexually, giving rise to many metazoites, which are also haploid. The metazoites go into the bloodstream and enter red blood cells, where they reproduce still more. Infected red blood cells break down and release metazoites. The result is recurring fever and chills, prime symptoms of malaria. Some metazoites in red blood cells develop into haploid germ cells which are also released into the bloodstream. When a female mosquito bites an infected human, she draws blood from that person, carrying along the germ cells. In the gut of the mosquito, the germ cells mature and fuse to form diploid *Plasmodium* zygotes. The diploid cells go through meiosis in the gut of the mosquito, producing sporozites. The cycle repeats when the mosquito bites another human, transmitting the infection (Figure 2).

Mosquitoes breed freely in standing water, and it has been known for centuries that areas close to foul-smelling marshes have a high incidence of malaria. In fact, the word malaria means "bad air" in Italian. Malaria is no longer endemic in southern Europe, largely as a result of draining marshes. Other deterrents to mosquito bites, such as insect repellents, protective clothing, and mosquito netting all have a long history of use in the fight against malaria. The World Health Organization (WHO) strongly urges the use of mosquito netting saturated with insect repellent to protect beds during nighttime hours when mosquitoes are active.

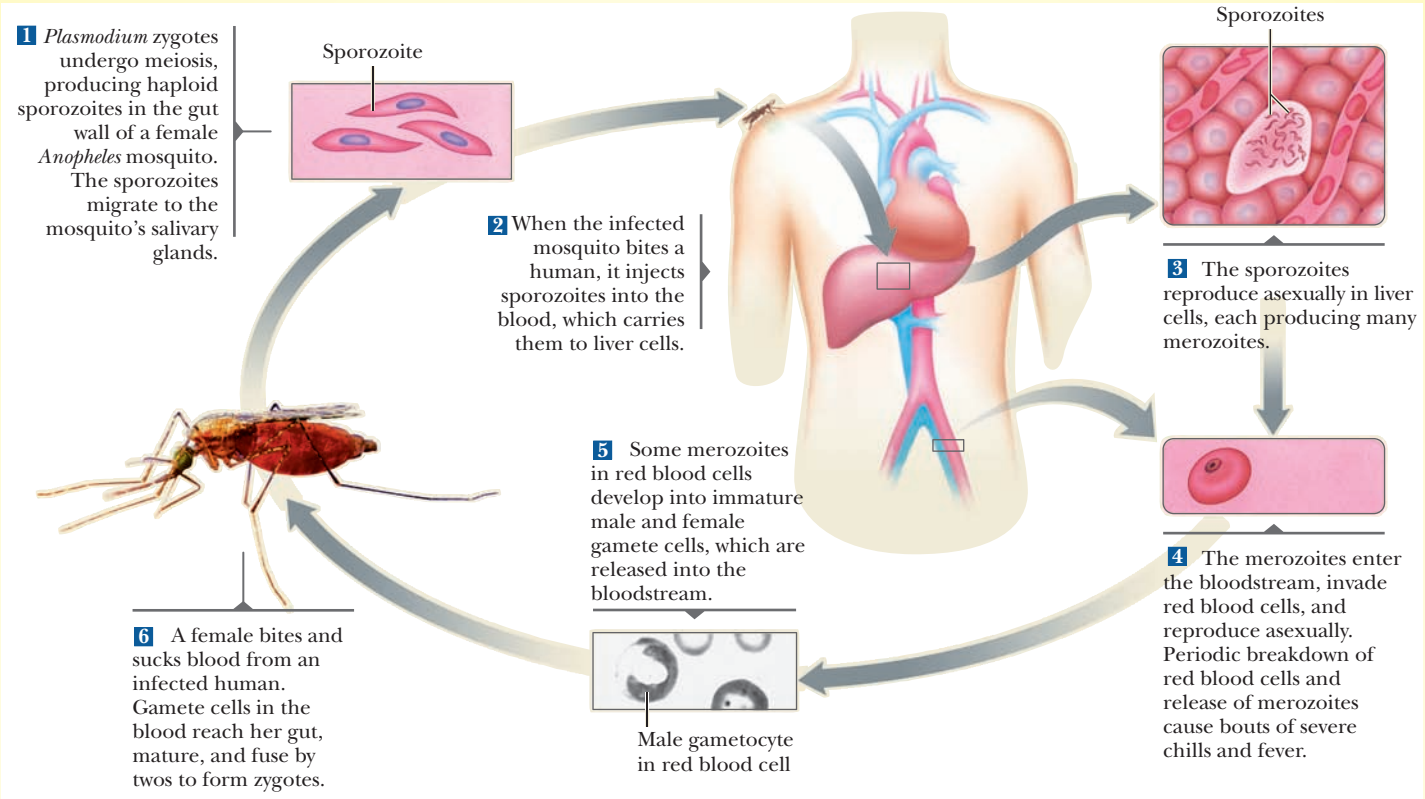


CDC

■ **FIGURE 1 A map from the CDC website about malaria.** The disease is widespread in the red areas and absent in the green ones. Specific links in the website provide up-to-date information about the yellow areas.

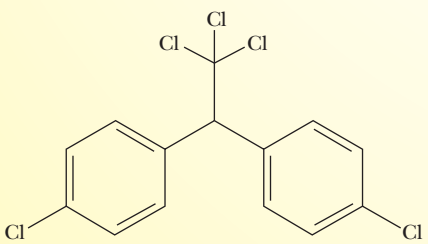
Cyrille Gibot/Alamy

■ The use of mosquito netting is a simple and effective way to prevent the spread of malaria.



■ **FIGURE 2 The life cycle of a malaria-causing *Plasmodium* protist.** *(From RUSSELL/WOLFE/HERTZ/STARR,* Biology, *1E. © 2008 Cengage Learning.)*

When a disease has an insect vector, the use of insecticides immediately comes to mind as an approach to controlling that disease. During World War II and in the years immediately following, DDT (dichlorodiphenyltrichloroethane) came into wide use to kill malaria-transmitting mosquitoes as well as insect vectors for other diseases. This compound is insoluble in water because of its nonpolar nature. As the structure shows, it has carbon-hydrogen and carbon-chlorine bonds, which are considered essentially nonpolar because of their small electronegativity differences, 0.4 and 0.5, respectively. As a result, DDT is highly soluble in fats, including adipose tissue of animals.



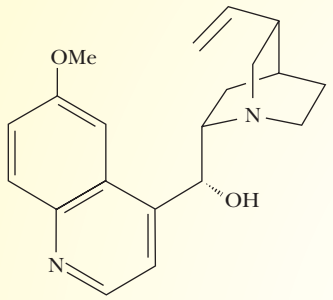
DDT

One of the problems with using DDT is that it tends to accumulate in animals, with concentrations increasing in animals higher in the food chain. DDT can appear in areas far from the site of original application because it can be carried by wind as a vapor. The accumulation in carnivores can have drastic consequences in the case of predators such as bald eagles and ospreys. When these birds metabolize DDT, a breakdown product interferes with the deposition of calcium in their eggshells. The shells crack under the weight of the adult birds in their nests, drastically reducing the number of young birds that actually hatch. DDT has been banned in the United States since the 1970s, but traces are widely distributed in all species, including human fat and breast milk.

The ban on DDT is not worldwide, and some restricted applications take place in the United States to protect public health. Heated controversy surrounds these restrictions, with a body of opinion that the non-use of DDT has led to the spread of diseases such as malaria and caused high death tolls. The discussion tends not to include an important point, namely the one of acquired resistance. In a given population of insects, there will always be some individual insects that are resistant to DDT. These are the ones that will survive to reproduce. The same principle leads to the rise of antibiotic-resistant pathogenic bacteria, with the constant need to develop new antibiotics. The effectiveness of DDT in controlling malaria is certainly reduced.

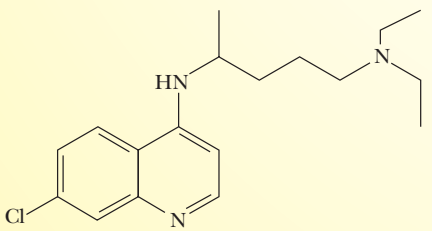
In addition to mosquito control in dealing with malaria, various medications have had wide use for prevention and treatment of this disease. One of the best known is quinine. This compound occurs in nature in the bark of the cinchona tree. The Quechua, indigenous inhabitants of Peru and Bolivia, discovered the properties of quinine in reducing fever and inflammation. Spanish missionaries in Peru adopted the use of quinine and introduced it in Europe in the 17th century, where it was successful in treating malaria. Cinchona bark became a valuable export for Peru, but that did not supply the demand for quinine. Cinchona trees were subsequently grown in

the Philippines and in what is now Indonesia, which became the world's leading supplier. During World War II, the United States and its allies were cut off from supplies of quinine by Japanese occupation of the areas in which cinchona trees were grown. Organic chemists succeeded in synthesizing quinine in the laboratory in 1944, but extraction from bark remains the most effective way to obtain quinine. By the 1940s, the search was on for other effective antimalarial agents.



Quinine

The next antimalarial to come into common use was chloroquine. Like quinine, chloroquine has a nitrogen-containing aromatic quinoline ring system. When chloroquine was first synthesized in the 1930s, it was feared that it might be too toxic to have medicinal use. Further testing under wartime pressure showed that it did have useful antimalarial properties and that it could be substituted for quinine, which was in short supply. Chloroquine kills the *Plasmodium* parasite by interfering with hemoglobin breakdown. When the hemoglobin from the blood of infected humans is degraded to the protein breakdown products and to heme, the heme cannot be allowed to accumulate. If heme is not removed by crystallization, it is toxic. Chloroquine interferes with the crystallization process, eventually killing the *Plasmodium*. The mode of action of quinine may be similar, but that point is not definitely established.



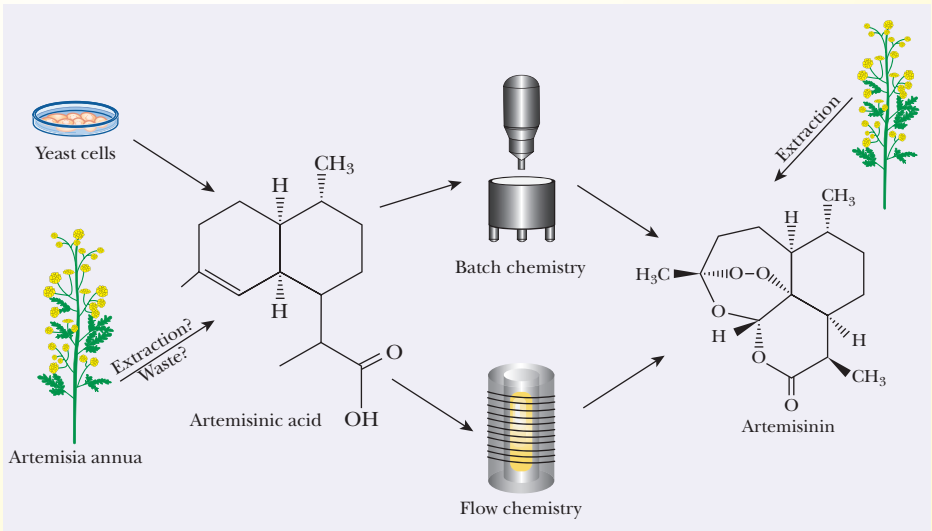
Chloroquine

Another antimalarial of plant origin has proved to be highly effective (see Biochemical Connections 22.2). This compound, artemisinin, can be extracted from *Artemisia annua*, a plant that has long been used in Chinese folk medicine. Artemisinin is in short supply because a typical plant contains only about 1% artemisinin. It is difficult to synthesize in the laboratory. Like quinine, it is more readily obtained by extracting it from the plant than by laboratory synthesis. That situation may be about to change. Two methods in development stages focus on converting a more readily available precursor, artemisinic acid, into artemisinin (Figure 3).

As shown in Figure 3, both processes require introducing a peroxide group with two oxygens bonded to each other into the precursor. The reaction requires energy, which is supplied in the form of light (a photochemical reaction). The batch process, which is at the stage where a factory is being built to carry it out, uses artemisinic acid from genetically engineered yeast cells. The flow process, which has so far been done only on a small scale, uses waste form the extraction of artemisinin from *Artemisia*. The light used to trigger the reaction can better penetrate the reaction mixture in a flow cell than is possible with a large batch vessel. It will be interesting to see which method eventually proves to be more effective. The efforts to produce more artemisinin could eventually save millions of lives every year.

Advances in the biological sciences have provided effective tools in obtaining information about the genome and the proteome of the mosquito vector and using that information to deal with malaria. *Anopheles gambiae* is the most common vector species. In 2002, its genome sequence was determined. Once the nucleotide sequence was established, the task was to assign the sequences to genes and families of genes. It was possible to use the complete genome of the fruit fly *Drosophila melanogaster* for comparison, because it was already available. *Drosophila melanogaster*, of course, is the species which has been used to establish many of the fundamental principles of eukaryotic genetics. More to the point, information about the proteome of both species was available for comparison. An analogy that is frequently used is that the nucleotide sequence of DNA is the script of a play, whereas the proteome (the entire set of proteins expressed by the genome at a given time) is the actual production of the play on stage.

The proteome comparison between *Anopheles* and *Drosophila* is particularly valuable in determining which genes play a more prominent role in *Anopheles* by giving rise to more proteins. Of particular



■ **FIGURE 3 Two possible ways of converting artemisinic acid to artemisinin.** The batch process uses genetically modified yeast cells to produce artemisinic acid for conversion to artemisinin. The flow process uses waste from the extraction of artemisinin from the plant; the waste contains more artemisinic acid than artemisinin originally found in the plant.

interest is the question, "Which genes are involved in blood feeding?" Both species have a number of serine proteases, which are effectors of innate immunity and similar processes that require protein hydrolysis, but *Anopheles* has about 100 more of this class of protein than does *Drosophila*. A number of classes of proteins are affected in the same way. One way to use this information is to select metabolic pathways connected with blood feeding and to target them with suitable agents. Receptors that allow the mosquitoes to select human prey based on odors represent another possible target and an opportunity to develop improved insect repellents. Yet another way is to find ways to inhibit *Plasmodium* development in the mosquito gut. The point here is to use the immune system of the mosquito to inhibit development of the parasite.

A number of proteins involved in the immune response in *Anopheles* are species-specific. Figure 4 shows a comparison of *Anopheles* proteins with similar proteins in *Drosophila*, expressed as a percentage. The bar on the left in each pair shows the genetic relationship of the total proteome with analogous proteins in related species. The blue sections show 1:1 correspondence in structure, and the yellow sections show close relationships. The green sections show species-specific proteins and ones with little homology to related species. *Anopheles* and *Drosophila* are similar as to the total proteome. The bar on the right in each pair shows the same information for immunity proteins. It is clear that *Anopheles* has a high proportion of species-specific proteins connected with the immune response, which present possible targets for intervention.

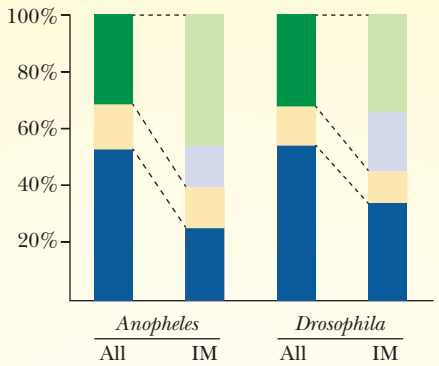


FIGURE 4 Comparison of immunity proteins in *Anopheles* and *Drosophila*, and comparison with total proteomes. The bar on the left in each pair (All) is the total proteome, and the one on the right in each pair represents the immunity proteins (IM). Green sections represent species-specific proteins (dark green) or little homology with other species (light green). Blue sections represent proteins that are similar in a number of species.

A disease as widespread as malaria is difficult to combat, but the very fact that it has so many different aspects presents a number of targets for researchers to approach. Malaria is not the scourge it was in the past, but more progress is an important goal in worldwide health care. It will be interesting to see what progress will take place in the next few years.

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Aging—Looking for the Biochemical Fountain of Youth

Soon after the dawn of humanity, people discovered that if they lived long enough, they experienced a gradual deterioration in health as they got old. Immediately after that, they realized they didn't like it. From that moment forward, humankind has been obsessed with finding ways to turn back the clock, or at least to stop it from advancing. In this book we have seen many references to practical applications of biochemistry that can lead to a higher quality of life and perhaps even a longer one. Most of these have been intuitive, such as maintaining a healthy lifestyle through diet, exercise, and avoidance of negative factors like smoking. However, we are never satisfied. This is the age of instant gratification—the age of Viagra, Rogaine, testosterone creams, and other drugs used to allow men to feel younger. Anybody would invest in a company that could develop a true antiaging pill—a fountain of youth in a bottle.

Although the exact causes of aging are still not clear, we believe it to be the gradual wearing out over time of the body's natural ability to maintain itself and repair damage. Logic dictates that natural selection cannot help our longevity, because the difference between living to 70 and living to 130 happens after the reproductive years, so there is no selective pressure for longevity. In other words, if there is a gene for longevity, it can only be selectively passed on if it affects reproductive success. Many scientists hypothesize that evolutionary changes lead species to prefer early development and procreation instead of maintaining a body into old age. Once the organism has reproduced, its genes are essentially immortal, although the vessel that carried them is not. Aging is believed to be driven by the lifelong accumulation of unrepaired cellular and molecular damage.

When We Think and Study Aging, What Are We Really Concerned About?

There are three major issues. One is maximum lifespan. This is the maximum time that any member of a species has lived. For humans this is about 120 years. Another is average life expectancy. This number has been going up for humans drastically for the last hundred years, although it is slowing considerably now. Average life expectancy has gone up over 30 years since the early 1900s mainly due to modern medicine. Diseases that killed people early in life and complications in child birth led to many deaths at a young age, which brought down the average life expectancy. So, the first is how long you would live if nothing killed you. The second is how long you really are likely to live given all environmental factors. A third consideration is the quality of life. Whether maximum life expectancy goes up or not, people today are experiencing a much higher quality of life as they age. When people say, "70 is the new 40," they mean that we now see septuagenarians being as active today as 40-year-olds were decades ago. The goal of gerontology is to improve health near the end of life, rather than to make people live 300 years. Doctors seek to increase "health span," the number of years free of chronic illnesses and other age-related issues.

Exercise and Aging

There can be no doubt that leading a healthy lifestyle can extend a person's life as well as make the available years more pleasant and productive. Fitness and diet can lead to a person avoiding many of the diseases that the elderly often succumb to, such as heart disease, stroke, and some forms of cancer. Being physically fit can slow the general decline we experience as we age. The earlier the fitness begins the better. Figure 1 shows how different types of fitness levels are related to the deterioration with aging.

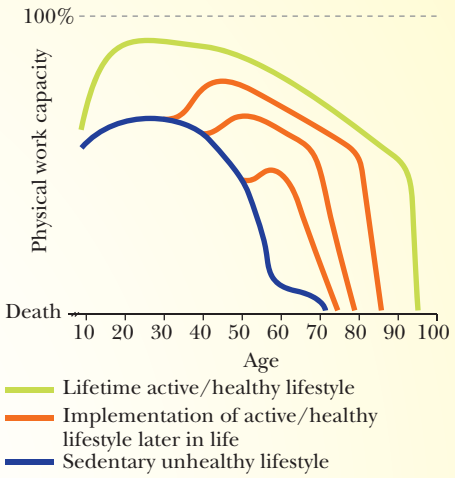


FIGURE 1 The effects of exercise on lifespan and quality of life. *(From Hoeger/Hoeger,* Fitness and Wellness, *10E. © 2009 Cengage Learning.)*

Figure 1 shows clearly that the earlier physically active lifestyles begin, the greater the person's ability to do physical work and the slower the decline is. The people who were active from age 10 years had a much higher overall work capacity. Equally important, at 85 they had the same work capacity as a 25-year-old sedentary person, and they made it past 90 before the most serious decline into death began. For many people that is the most important statistic. When people wonder whether they would really want to live for a hundred years, their answer would undoubtedly depend on what those years looked like. As a famous comedian once said, "Do I really want another 20 years of wearing adult diapers?" Most people, if given the

choice, would like to live a long time and would probably choose the green curve in the figure, where they are relatively healthy well into their old age, and then a quick decline rather than a slow and lingering one that burdens themselves and their families.

Can Longevity Be Increased with Chemistry?

But what if we could have increased longevity and quality of life? We discovered more than 70 years ago that calorie restriction (CR) is associated with increased longevity in life forms as varied as yeast and rodents. Recently, primates were added to that list. In some species, restricting caloric intake by 30% compared to normal levels was shown to increase life span by 30% or more. This technique is still the only absolutely proven method of extending life span, other than not smoking and avoiding the more obvious dangerous behaviors. In addition to the life span extension, CR leads to a higher quality of life and forestalls many diseases, such as cancer, diabetes, inflammation, and even neurodegenerative diseases. Many mechanisms for this longevity increase have been suggested, including general health benefits of weight reduction and specific improvements in DNA management due to lower levels of oxidative compounds that are created as by-products of metabolism. However, about 15 years ago researchers began to pinpoint a family of genes in the yeast *Saccharomyces cerevisiae* that seemed to be at the center of these increases in longevity due to CR. The best characterized of these genes is SIR2 in yeast. SIR2 is a member of a family of genes called the sirtuin genes, and evidence indicates that they are key regulators of the longevity mechanism. Their mode of action is based on fundamental changes in the organism's metabolism, especially the insulin-signaling pathways. In yeast and in roundworms, genetic manipulations that doubled the number of SIR2 genes increased life span by 50%!

The protein product of the mammalian version of the SIR2 gene is a protein called SIRT1. It is a stress-induced protein deacetylase that is dependent on NAD^+. It regulates cell survival, replicative senescence, inflammation, and metabolism via the deacetylation of histones (Chapter 10). CR is a biological stressor like natural food scarcity. SIRT1 seems to be at the center of a generalized response to stress that primes the organism for survival. As Figure 2 shows, SIRT1 in mammals occupies a pivotal role in longevity through improved DNA stability, increased repair and defense, prolonged cell survival, enhanced energy production and use, and other coordinated stress responses.

Mice that have been engineered to lack SIRT1 do not show the longevity increase associated with CR. Furthermore, doubling the number of SIRT1 genes in an organism renders it unresponsive to calorie restriction. Thus, it is now generally accepted that calorie restriction promotes longevity by activation of sirtuins in general and SIRT1 in particular.

Of course, humans prefer not to live a life of deprivation in order to reap the benefits of life-span extension, and thus, the search for a stimulator of SIRT1 was on. One of the first compounds found that is a natural activator of sirtuins is a small molecule called resveratrol, a polyphenol that is present in red wine and made by many plants when stressed. Feeding resveratrol to yeast, worms, or flies, or placing them on a CR diet, extends their life spans about 30%, but only if they possess the SIR2 gene. Resveratrol was found to increase SIRT1 activity 13-fold. As far as a small molecule that might increase longevity, nothing could be more attractive than resveratrol, especially to wine drinkers. Increased levels of SIRT1 in mice and rats allow some of the animals' cells to survive in the face of stress that would normally trigger their programmed suicide. It does this by regulating several other key cellular proteins, such as p53 (Chapter 14), NF-kB, HSF-1, FOXO1, 3, and 4, and PGC-1a (Figure 3). In addition, SIRT1 is stimulated by increased ratios of NAD^+/NADH, a situation that arises when respiration is increased, as happens with fasting. Thus, SIRT1 is believed to act as both a sensor of nutrient availability and a regulator of the liver's response. SIRT1 has been linked to regulation of insulin and insulin-like growth factor. As seen in Chapter 14, insulin is known to play an important role in the general metabolic state of the organism.

The discovery of the sirtuins and of the effect of CR and resveratrol led to further research into aging and longevity. Unfortunately, in the last few

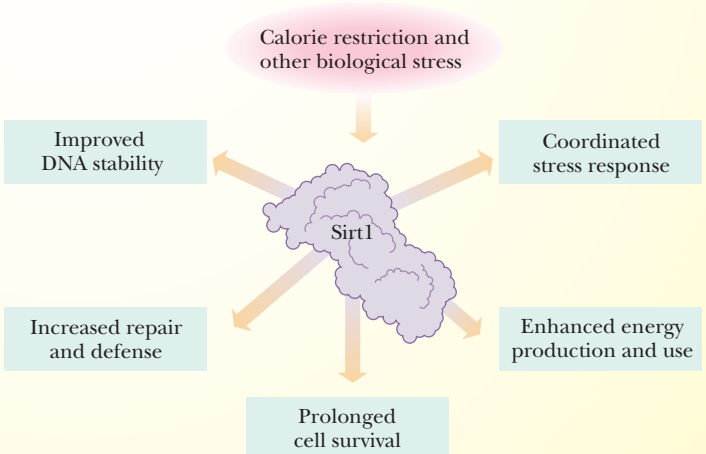


FIGURE 2 SIRT1 and its putative relationship to health and longevity. The SIRT1 enzyme appears to be responsible for the health and longevity-enhancing effects of calorie restriction in mammals. Food scarcity and other biological stressors trigger increased activity by SIRT1, which in turn alters activities within cells. By boosting manufacture of certain signaling molecules, such as insulin, SIRT1 may also coordinate the stress response throughout the body. *(Reprinted by permission of* Scientific American, *"Unlocking the Secrets of Longevity Genes" by David A. Sinclair and Lenny Guarente, March 2006.)*

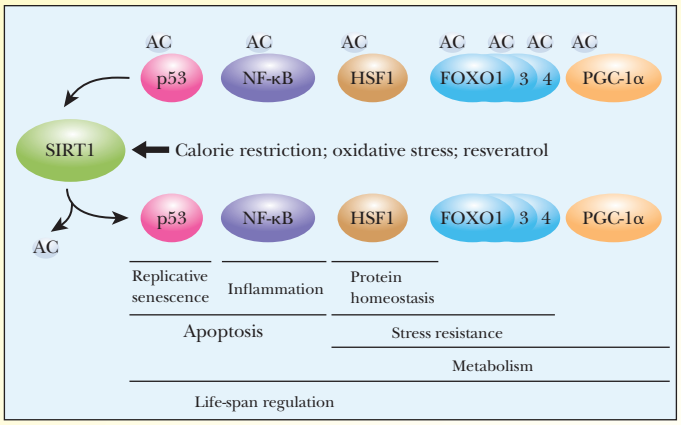


FIGURE 3 **SIRT1 is an enzyme that deacetylates several key transcription factors that affect metabolism and aging.** *(Based on Saunders, L. R., and Verdin, E. (2009). Stress response to aging.* Science *323, p. 1021. Copyright © 2015 Cengage Learning®.)*

years, several researchers, including one who did some of the original research, have had nagging doubts due to their inability to repeat key parts of the experiments. Some experiments have shown that resveratrol does nothing to help yeast cells live longer. Other studies showed that CR extended lifespan in yeast even when the SIR2 gene is deleted, which is in conflict with the original theory that CR is affecting SIR2.

Several important signaling pathways have been found to play a role. A drug called rapamycin was found to increase life span in mice. Its direct target is a protein that was given the name mammalian target of rapamycin (mTOR). Both CR and rapamycin lower the activity of the mTOR enzyme, as shown in Figure 4.

The mTOR enzyme activates a ribosomal S6 protein kinase (RSK), called S6K1, which phosphorylates S6 ribosomal proteins. The RSKs modulate mRNA translation and protein synthesis in response to mTOR signaling. It has been shown that longevity is increased by inhibiting the mTOR enzyme, which in turn inhibits the S6K1 enzyme. Another protein kinase, AMPK, appears to be stimulated by the process.

Although we are decades away from seeing a true longevity pill, the studies referenced here indicate promise that such a compound can be found. As is often the case, it should be much easier to find the treasure when we are sure the treasure exists. Both mTOR and S6K1 can be modified by small molecules, as we have seen in the case of rapamycin. Rapamycin has been shown to reduce adiposity in mice, at least in the short term. Why then have we not seen rapamycin on the shelves at our local pharmacy?

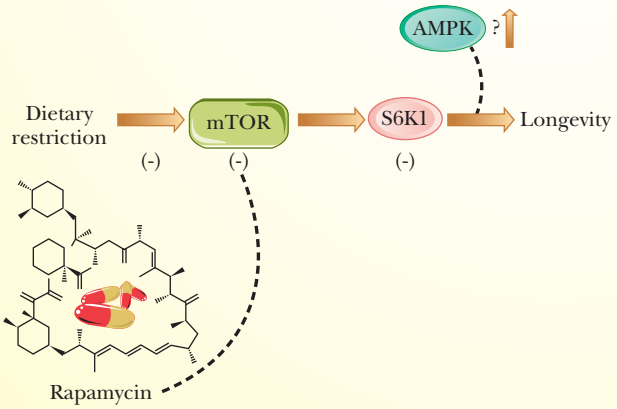


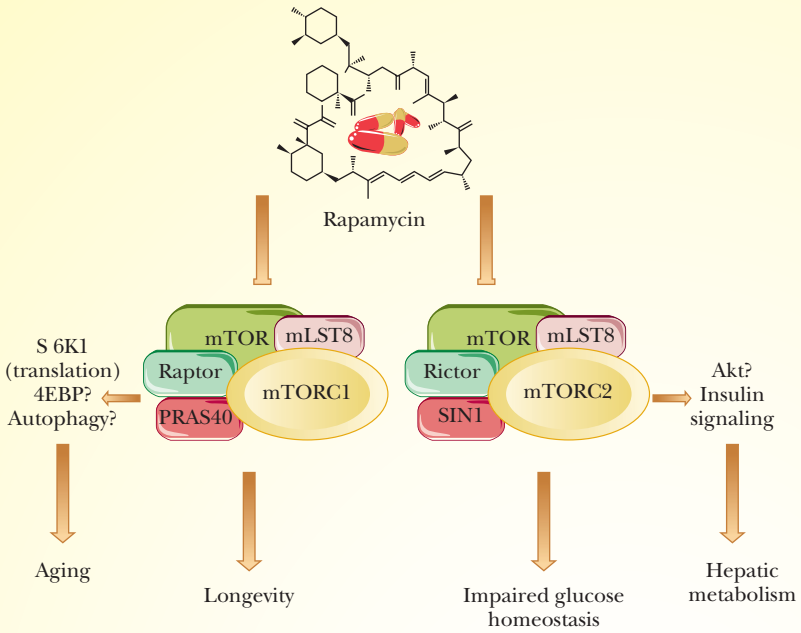
FIGURE 4 **Chemical basis for longevity.** Both dietary restriction and the drug Rapamycin inhibit the protein mTOR. When mTOR is inhibited, it inhibits its production of S6K1, which leads to increased longevity. In a poorly understood mechanism, the protein AMPK is stimulated by the same process. *(Based on Kaeberlein, M., and Kapahi, P. (2009). Aging is a RSKy business.* Science *326, p. 55. Copyright © 2015 Cengage Learning®.)*

The reason is that we have a long way to go before we truly understand this process. For one thing, scientists are concerned about side effects. A known side effect of rapamycin when used long term is immune suppression. Furthermore, evidence exists that attempts to prolong life also often have the consequence of stimulating cancers. Several studies of cancer cell lines have shown they have significantly greater levels of sirtuins than regular cells. Thus, stimulating the longevity of cells doesn't work if we stimulate the wrong kind of cells. Rapamycin has also been implicated in mice and humans with glucose intolerance and insulin resistance. These side effects, if let go long enough, could outweigh the benefits to longevity.

Scientists are currently looking for ways to uncouple the positive effects on longevity with the negative effects on glucose homeostasis that rapamycin produces. The answer may lie in the details of what the mTOR protein does. It has been found that mTOR is involved in two different protein complexes, as shown in Figure 5.

mTOR is involved in a complex called mTORC1, which regulates pathways involved in autophagy, mRNA translation, and other cellular pathways. It is also associated with mTORC2, which regulates insulin signaling. Inhibiting mTOR via rapamycin inhibits both pathways, although with different effects—increasing longevity when mTORC1 is inhibited and impairing glucose metabolism when mTORC2 is inhibited. Thus, scientists continue to look for a small molecule that can have the same effect on mTORC1 as rapamycin but which will not inhibit mTORC2.

In summary, life expectancy can be increased by maintaining a healthy lifestyle and avoiding activities that can kill you, which should be a no-brainer, but somehow is not in many people. However, the knowledge gained from the studies on sirtuins and mTOR in the last decade has been the first indication that we may yet be able to take control of our own longevity destiny, including maximum life span, albeit sometime in the future.



■ **FIGURE 5 Rapamycin affects two different pathways.** *Adapted from Hughes, K. J., and Kennedy, B. K. (2012). Rapamycin paradox resolved.* Science *335, 1578.*

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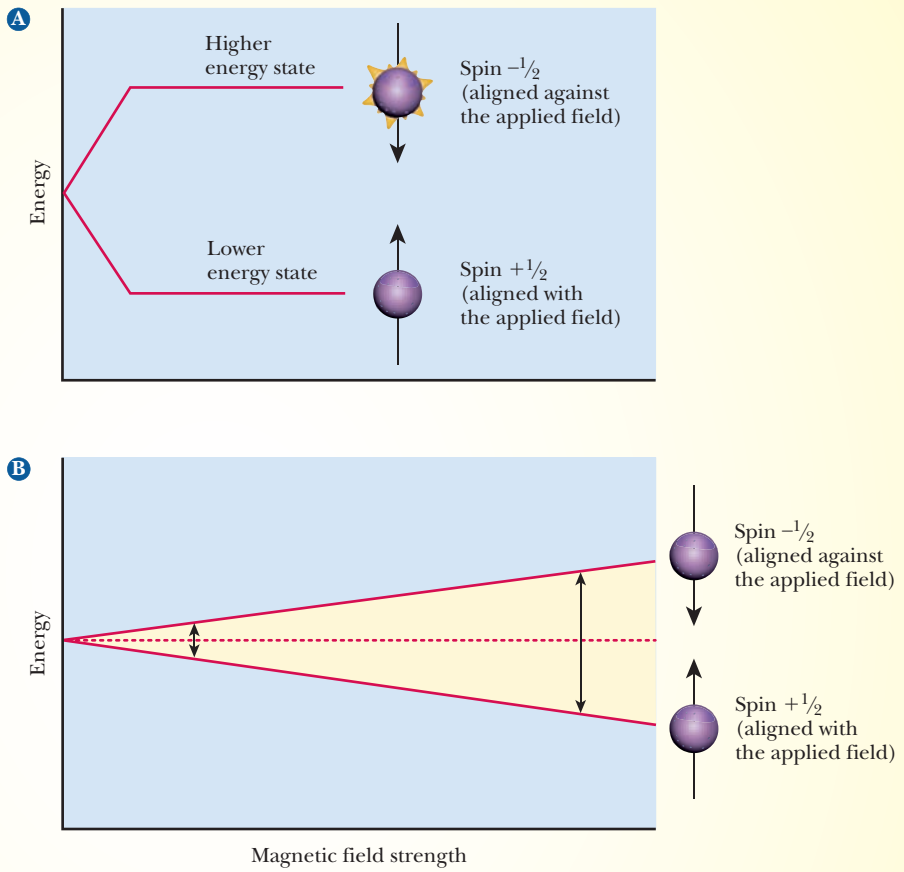
Proteins and Magnets: Nuclear Magnetic Resonance in Biochemistry

Many of the readers of this book have encountered nuclear magnetic resonance (NMR) spectroscopy in an organic chemistry course. This technique is also assuming a large role in biochemistry, rivaling its importance to organic chemists. NMR operates on the same physical principle as magnetic resonance imaging (MRI) in wide use in clinical practice. The different names are used to avoid confusion that might arise from the word "nuclear," which is frequently associated with radioactive material. Radioactive isotopes are widely used clinically for diagnosis and treatment (nuclear medicine). However, all atoms have nuclei, and many more nuclei are stable than those that undergo radioactive decay.

The property of atoms that makes NMR possible is known as nuclear spin. When a charged particle, such as an atomic nucleus, spins, it creates a magnetic field associated with the motion of electric charge. Each kind of nucleus has a characteristic spin quantum number, designated as ℓ. (Quantum numbers are a concept from general chemistry, where they are used to describe aspects of atomic structure.) Some have a spin quantum number of 0, some have half-integral values, and some have integral values, depending on the number of protons and neutrons that comprise each nucleus. The spin quantum number determines the number of spin states, which in turn are the basis of NMR spectroscopy. The following table shows the number of spin states for some important nuclei.



■ **FIGURE 1** An applied magnetic field brings about a separation of energy in spin states of nuclei such as ^{1}H and ^{13}C with spin quantum number = ½. Part (a) shows the separation in energy when spins are aligned with or against the magnetic field. Part (b) shows how the energy difference depends on the strength of the applied magnetic field. *(From BROWN/FOOTE, ORGANIC CHEMISTRY, 2E. © 1998 Cengage Learning.)*

Spin Quantum Numbers and Nuclear Spin States for Selected Isotopes of Common Elements

Element	^{1}H	^{2}H	^{12}C	^{13}C	^{14}N	^{15}N	^{31}P
Nuclear spin quantum number (ℓ)	1/2	1	0	1/2	1	1/2	1/2
Number of spin states ($2\ell + 1$)	2	3	1	2	3	2	2

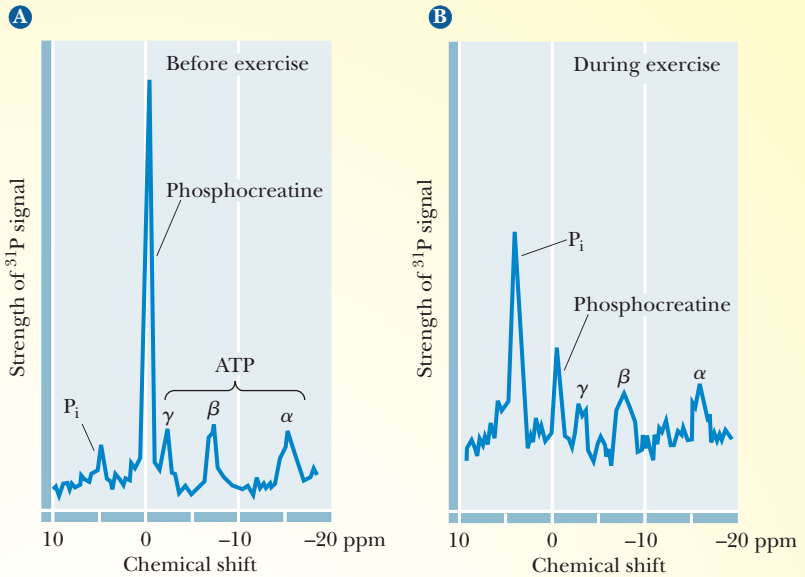
All forms of spectroscopy depend on transitions between energy levels in the sample molecules. In this case, the various energy levels are the different spin states in an applied magnetic field. Note particularly the point about the applied magnetic field. In the absence of an external magnetic field, the spin states have the same energy. When the magnetic field is applied, the spin states interact differently, giving rise to different energies. (Of course, if there is only one spin state, there is no separation and

no possibility of a transition.) The energy separation depends on the strength of the magnetic field. Figure 1 shows how this separation comes about and how it is dependent on magnetic field strength. Note that this example is for nuclei with half-integral spin quantum numbers. Integral spins give rise to more energy levels, with the possibility of more transitions and, consequently, of more complex spectra. As a result, nuclei with half-integral spins are used in NMR in preference to those with integral spins.

Like all forms of spectroscopy, NMR measures the interaction of a sample with electromagnetic radiation in a given wavelength range, whether ultraviolet, infrared, or radiofrequency (RF). For NMR, the appropriate region of the spectrum is in the radiofrequency region. In optical spectroscopy (ultraviolet or infrared), a sample is placed in a path between the light source and a detector. The presence of a magnetic field in NMR literally adds another dimension. The radiation source (an RF transmitter) is positioned at a 90-degree angle to the directions of the magnetic field, and the detector (an RF receiver) is placed at a 90-degree angle both to the direction of the magnetic field and the RF transmitter.

For more than 50 years, scientists have worked to improve NMR spectroscopy to the point where it is possible to study molecules as large and complex as proteins. Two factors are particularly important in making it possible to obtain detailed NMR spectra of molecules as complex as proteins. The first is the invention of superconducting magnets. It is now possible to use magnetic fields more than an order of magnitude more powerful than those used 50 years ago. Because of the increase in field strength, peaks in spectra are further apart and easier to analyze. The second factor is the increase in computing power that allows researchers to process raw data by using the mathematical process called Fourier transformation. Large amounts of data can be processed quickly, allowing for greater accuracy and reduction in random errors. Most NMR spectra obtained at present are Fourier transform NMR (FT-NMR) spectra.

The majority of NMR spectra are proton NMR spectra, which depend on transitions of the usual isotope of hydrogen. It is possible to use any nucleus with a half-integral spin with some qualifications. The isotopes ^{13}C and ^{15}N have low natural abundance, but it is possible to obtain spectra based on them by enrichment techniques. In addition, ^{31}P, with half-integral spin is the usual isotope of phosphorus, and it can be used in applications in which phosphorus plays a role. Figure 2 shows an example of phosphorus NMR that shows a connection between NMR spectroscopy and clinical MRI.

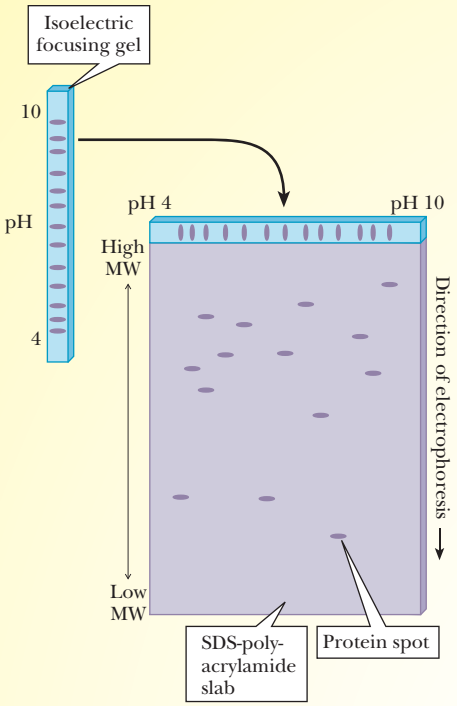


■ **FIGURE 2** Phosphorus NMR spectra of the forearm muscle of a living subject in real time. Part (a) shows the spectrum before exercise, and part (b) shows the result during exercise. Note that there are three separate peaks for the three phosphorus atoms (α, β, and γ) of ATP. *(From GARRETT/GRISHAM,* Biochemistry, *4E. © 2009 Cengage Learning.)*

We saw in Chapter 15 that phosphorus compounds are important in bioenergetics. Figure 2 shows phosphorus NMR spectra of the forearm muscle in a living human subject (a) before and (b) during exercise. Five peaks are visible in each spectrum: three for the three phosphorus atoms of ATP, and one each for phosphocreatine and phosphate ion (P_i). The term "chemical shift" refers to the fact that each of the phosphorus atoms is in a slightly different environment in the magnetic field because of the bonding pattern in each of these compounds. Each of the two parts of the figure indicates the presence of the electronic environment (chemical shift) for each phosphorus atom, with the height of the peak indicating the relative amount.

Larger molecules such as proteins will have spectra that are much more complicated than the previous example, so more information is needed to analyze the data. Separating the information in a spectrum into usable form is done by adding dimensions to the data analysis. An analogous approach can be seen in Section 5-3, where a sample that contains a mixture of proteins is subjected to electrophoresis in one dimension to achieve a partial separation and then to subject the resulting group of partially separated proteins to another electrophoresis technique at 90 degrees to the original. Figure 3 shows the general outline of how the two-dimensional separation is achieved.

Another analogy that has been used is that a conventional NMR spectrum of a protein is like a multivolume book that has been compressed to a single line of text with all the words mixed together. If the single line (one dimension) could be expanded to a page (two dimensions), that would be a step in the right direction, but most words would still be mixed together. A third dimension would expand the information to a book, and significant portions of the message would become intelligible. A fourth dimension would result in the multivolume set with the message becoming easily readable. NMR experiments in



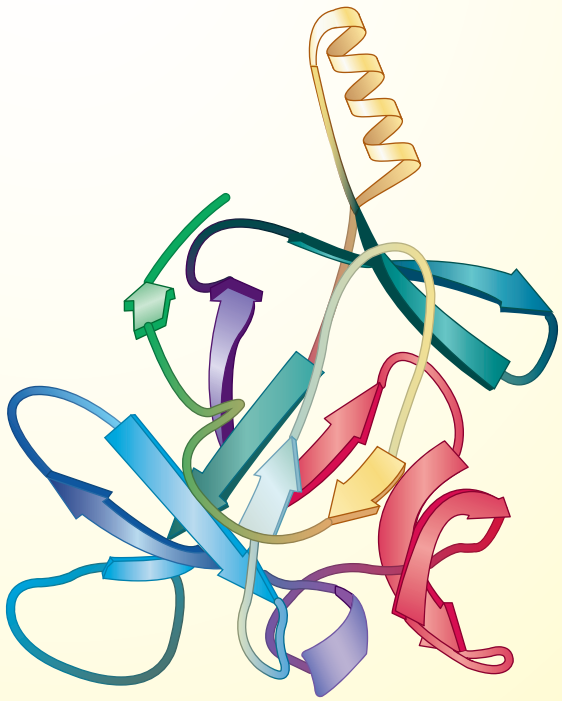
■ **FIGURE 3** **Two-dimensional electrophoresis.** A mixture of proteins is separated by isoelectric focusing in one direction. The focused proteins are then run using SDS-PAGE perpendicular to the direction of the isoelectric focusing. Thus the bands that appear on the gel have been separated first by their isoelectric points and then by size.

two, three, and four dimensions have been done, all making it possible to analyze ever more complex spectra.

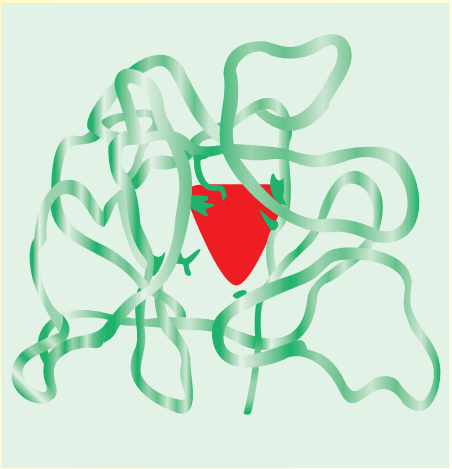
The actual process of multidimensional analysis of NMR spectra is highly mathematical and depends on the Fourier transform method. Adding dimensions to NMR spectra ultimately depends on setting up the experiment so that the Fourier transform can be done a number of times. The process is quite complex, and we are not going to go into details. The signal is analyzed by Fourier transformation and plotted on one axis of a graph (one dimension). Each time a different Fourier transform of the signal is done adds another axis to the graph. The final results show interaction between nuclei in the magnetic field, and consequently, identify the nuclei that are close to each other, which is key structural information. Model building based on this these results can give structures of proteins that rival the results of X-ray diffraction.

During the 1980s, a number of experiments were done with two-dimensional (2D) NMR of small proteins, ones that contained fewer than 100 residues. These studies produced structures similar to those obtained by X-ray crystallography. The desire to study larger proteins led to the addition of the third and fourth dimensions. In a classic 1991 paper cited in the bibliography, researchers from the National Institutes of Health (NIH) described the results of multidimensional NMR on interleukin-1β, a 153-residue protein that plays an important role in inflammation. They obtained proton (^{1}H), ^{13}C, and ^{15}N NMR spectra. The data processing to combine the spectra for each kind of nucleus to give 2D spectra was done, followed appropriate combinations to give the three- and four-dimensional (3D and 4D) results. Combining all the spectra allowed the researchers to arrive at a structure. Their results were comparable with those obtained by X-ray diffraction. This structure can be found in the Protein Data bank under the URL http://www.rcsb.org/pdb/explore/explore.do?structureId=6I1B. The structure is shown in Figure 4.

Another NMR study on the structure of interleukin-1β shows some of the unique features of protein structure determination by NMR compared to what can be obtained by X-ray crystallography. The important difference between the two methods is that the NMR studies we have discussed use samples that are in solution, whereas X-ray crystallography, by definition, uses crystalline samples. Not all proteins can form crystals, which immediately require a solution-based method to determine the structure of such proteins. In addition, even crystalline samples contain a fair amount of the liquid (the "mother liquor") in which the protein was dissolved and from which it was crystallized. The mother liquor usually contains salts, such as ammonium sulfate, and crystals such as these have water molecules called water of hydration. If a water of hydration is tightly bound in a fixed position, it will appear in the final crystallographic structure. If it is loosely bound without a fixed position, it will not appear in the structure. However, since NMR solution studies take into account the fact that molecules in solution are in constant motion, a water molecule that is associated with a protein can be detected, even if it is not tightly bound in a fixed position (Figure 5). The NMR methods developed for proteins of this size gave a structure showing loosely bound water in the hydrophobic cavity of interleukin-1β. Until that time, the presence of that water was not known.



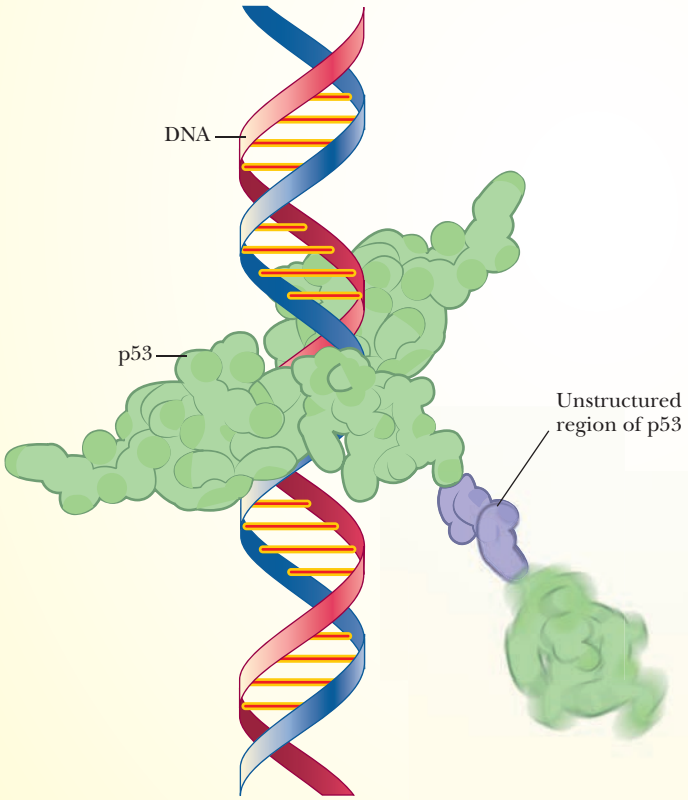
■ **FIGURE 4** Structure of interleukin-1β determined by solution NMR. (*From the Protein Data Bank.*)



■ **FIGURE 5** Ribbon diagram of interleukin-1β. Rear view showing protein backbone in green and bound water in red. *(Adapted from Ernst, J. A., et al. (1995). Demonstration of positionally disordered water within a protein hydrophobic cavity by NMR.* Science *267, 1815.)*

If loosely bound water can be detected, the question arises whether conformational mobility can be detected within the protein molecule itself. The answer is that it is indeed possible with solution NMR, but not with X-ray crystallography. Conformational mobility is an integral part of the mode of action of some proteins. The tumor suppressor protein p53 is a prime example. It was known from earlier NMR studies that this protein is a tetramer, but more recent work determined that the mobile parts interact with DNA. Binding of the p53 protein to DNA triggers the action of DNA-repair enzymes (Figure 6). Partial unfolding of monomers of the protein allows the binding to take place as it should. This work on the unstructured region of the protein could not have been done with any other method.

The power of NMR spectroscopy to deal with questions of biochemical importance is limited only by the ingenuity of the researchers who devise the experiments. The computer algorithms for obtaining spectra and analyzing the results are constantly being improved. For example, the paper by Raman et al. cited in the bibliography describes the use of highly sophisticated computer algorithms to deduce protein structures from data about the backbones, but not the side chains. This method was used with data from proteins of known structure. The results of this method were comparable to those obtained by earlier methods. It will be interesting to see what the future may hold as biochemical applications of NMR spectroscopy improve still further.



■ **FIGURE 6** The unstructured regions of p53 allow the protein to wrap itself around the double helix of DNA. Part of p53 is already bound to the double helix. The unstructured portion of p53 (shown in purple) has enough conformational mobility to allow the rest of p53 (green blur) to move into position to bind to other parts of DNA. The part of p53 that is not yet bound is highly mobile. *(AXS Biomedical Animation Studio, Inc.)*

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G-Protein–Coupled Receptors

When our bodies respond to external stimuli such as light or odor, or when we experience the fight-or-flight response, the widely distributed class of proteins known as G-protein–coupled receptors (GCPRs) play a central role. The pivotal position in life processes of these receptors received a lot of media attention in the fall of 2012 with the announcement of the award of the Nobel Prize in Chemistry to Brian Kobilka of Stanford University and Robert Lefkowitz of Duke University for their work on these proteins. What are the characteristics of these proteins that makes them so important?

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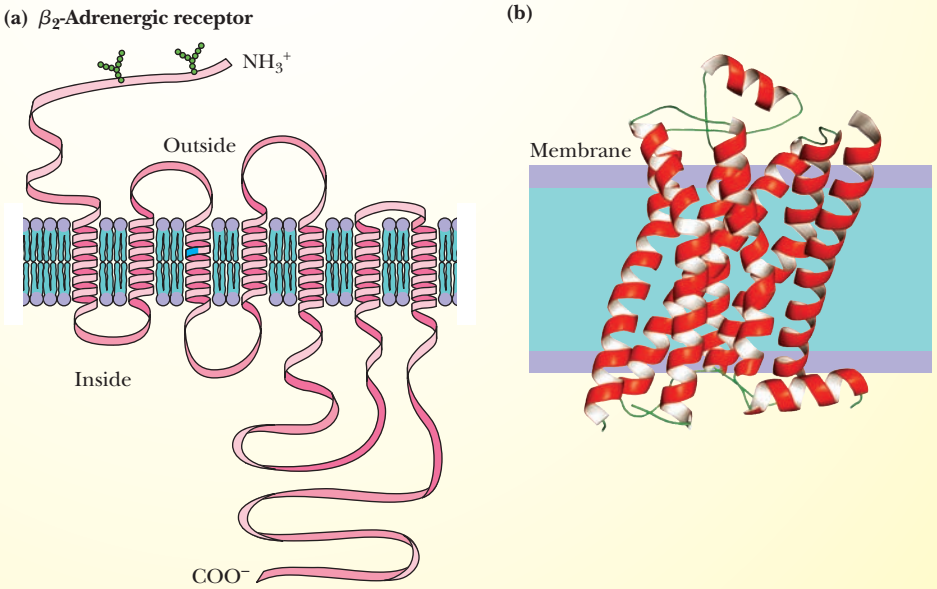
■ Brian Kobilka

JONATHAN NACKSTRAND/AFP/Getty Images/Newscom

■ Robert Lefkowitz

These receptor proteins occur in cell membranes and are coupled to G proteins on the inner side of the cell membrane. (See Biochemical Connections 8.2 and Section 24-3.) All GCPRs span the membrane and consist of seven α-helical segments. They are also called 7-transmembrane segment proteins (7-TMS). Rhodopsin, the key protein in vision, and the α- and β-adrenergic receptors, both of which bind the hormone epinephrine, are examples of GCPRs. As an example, Figure 1 shows the structure of a β-adrenergic receptor.

When a signaling molecule binds to a GPCR, as shown in Figure 2, it sets off a series of events that lead to transmission and amplification of the signal within the cell. The binding event activates the G protein; thus the name G-protein–coupled receptor. The result can be inhibitory or stimulatory, depending on the specific kind of receptor. Whether the binding is inhibitory or stimulatory, it brings about dissociation of the trimeric subunit structure of the G protein. The subunits are designated α, β, and γ. The β and γ subunits remain bound to each other. The α subunit goes on to signal the next step in the cell, such as inhibition or activation of a specific enzyme. In the process, an exchange of GDP bound to the α subunit for GTP takes place. The bound GTP is slowly hydrolyzed by the α subunit. This GTPase activity gives rise to the name G protein. These reactions are described in detail in Chapter 24. The wide range of effects depends on the fact that a number of different receptors exist, all with several subtypes. A number of types of G proteins exist as well, with most of the variation in the α subunit. The various combinations of receptors and G proteins can give rise to a

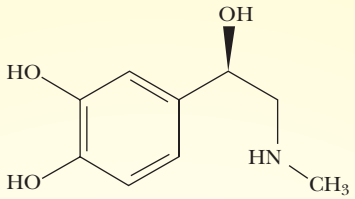


■ **FIGURE 1 Structure of a β-adrenergic receptor.** (a) Schematic diagram of the seven transmembrane helical segments. (b) The main features of the structure of a β-adrenergic receptor. *(From GARRETT/GRISHAM,* Biochemistry, *4E. © 2009 Cengage Learning.)*

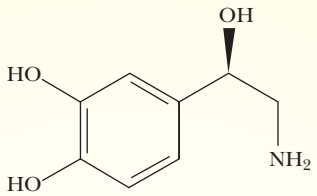
number of responses. (The discovery of G proteins led to the award of the 1994 Nobel Prize in Physiology or Medicine to Alfred Gilman of the University of Texas Southwestern Medical Center and Martin Rodbell of the National Institutes of Health.)

The number of GPCRs runs into the hundreds, with many roles in the body. They are grouped into classes depending on similarity of amino acid sequence and functional specificity. Of this enormous group, we will concentrate on the two main kinds of receptor for the neurotransmitter hormone epinephrine, the α- and β-adrenergic receptors. These receptors bind to catecholamines, namely epinephrine and its analog norepinephrine, the hormones of the fight-or-flight response.

The α- and β-adrenergic receptors have a number of subgroups. The members of these subgroups interact with different kinds of G proteins and have different effects. The α_1 receptor increases intracellular calcium ion concentration and stimulates smooth muscle contraction.



Epinephrine

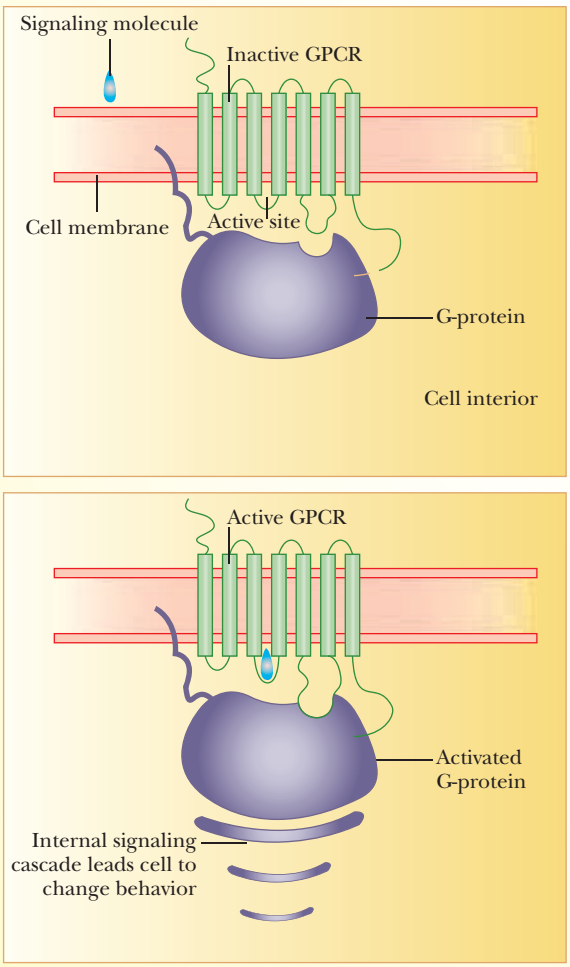


Norepinephrine

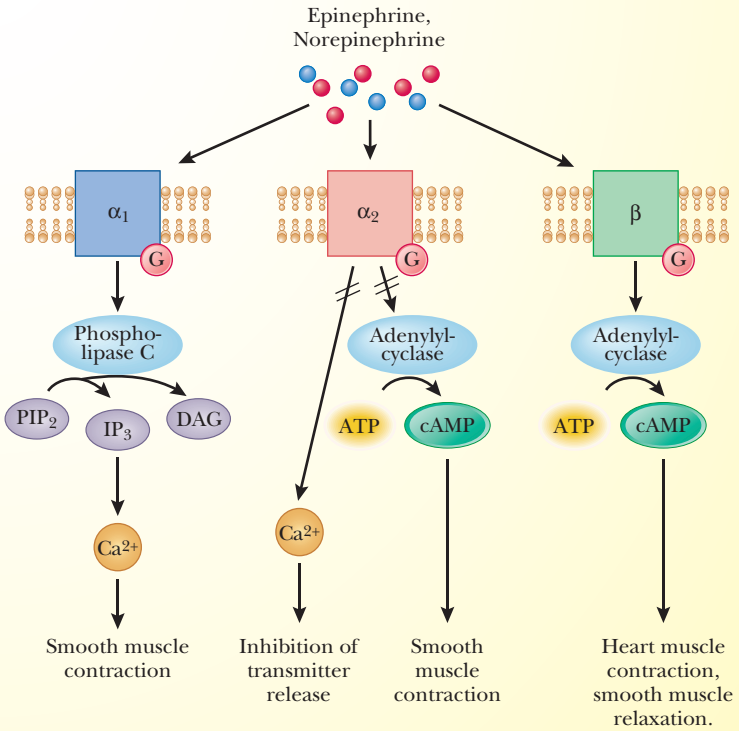
The α_2 receptor group plays a number of roles, many of them connected with the gastrointestinal tract. The β receptors are divided into three main types (Figure 3); the results of their operation range from heart muscle contraction to smooth muscle relaxation.

A good way to understand GPCRs, their structure, and their many functions, is to look at the methods that were used to determine their properties. Here are some of the questions we can ask. What genes encode these proteins? What is the amino acid sequence specified by these genes? What is the three-dimensional structure of GPCRs? How does the structure of these receptors determine their functions?

The cloning, sequencing, and expression of the gene coding for the human platelet α_2-adrenergic receptor was an important early step in understanding relationships among these receptors. Using the amino acid sequence of the purified protein as a guide, Kobilka, Lefkowitz, and their colleagues synthesized corresponding oligonucleotide probes. The probes were used to screen a human genomic DNA library,



■ **FIGURE 2** The binding of a signal molecule to a GCPR leads to activation of a G protein, which, in turn, leads to an effect within the cell by a cascade mechanism.



■ **FIGURE 3** **A summary of the effects of binding of catecholamines to adrenergic receptors.** See Section 24-4 for a description of the reactions of phospholipase C and adenylyl cyclase.

using techniques described in detail in Chapter 13. The gene that was recovered codes for a protein 450 amino acid residues in length. The next step was to use hydrophobicity analysis to predict regions of the sequence that were likely to represent transmembrane helical segments. This analysis depends on the tendency of amino acids with hydrophobic side chains to be in locations removed from the aqueous medium and the corresponding tendency of amino acids with polar side chains to be in contact with the aqueous medium. The table shows the hydrophobicity index for the standard amino acids. A positive number indicates a side chain that is likely to be in the hydrophobic portion of a protein, and a negative value indicates a side chain that will be found in contact with the aqueous medium.

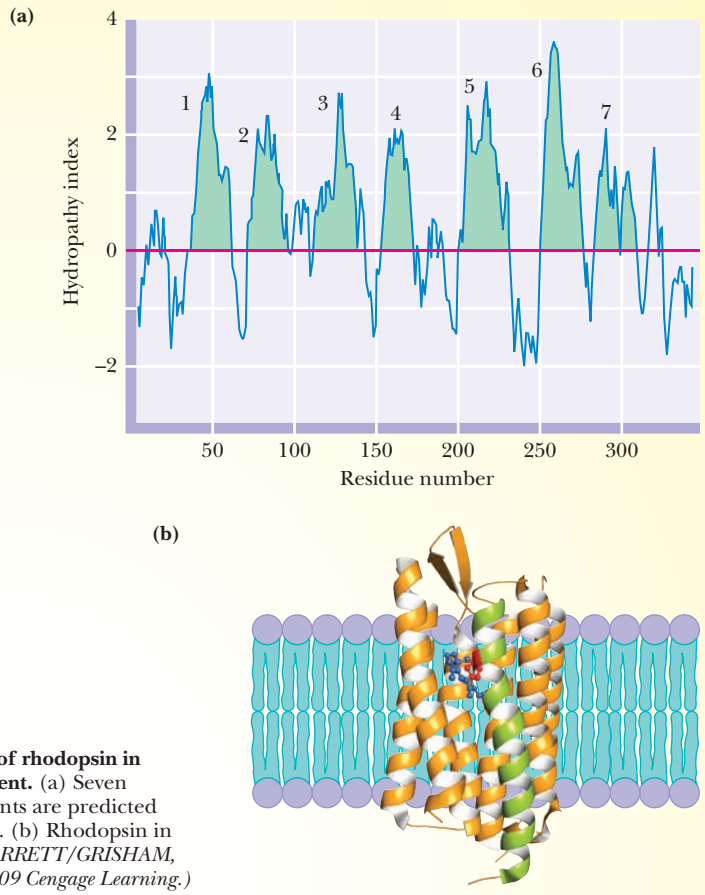
Results were available from a comparable analysis of the visual protein rhodopsin, which is also a GPCR (Figure 4).

Sequence analysis of the α_2-adrenergic receptor showed a similar pattern of seven helical regions, with a good deal of sequence homology with rhodopsin and other GPCRs. Expression of the cloned gene gave a protein that showed the same ligand behavior as the protein

Hydropathy Scale for Amino Acid Side Chains in Proteins*

Side Chain	Hydropathy Index
Isoleucine	4.5
Valine	4.2
Leucine	3.8
Phenylalanine	2.8
Cysteine	2.5
Methionine	1.9
Alanine	1.8
Glycine	−0.4
Threonine	−0.7
Serine	−0.8
Tryptophan	−0.9
Tyrosine	−1.3
Proline	−1.6
Histidine	−3.2
Glutamic acid	−3.5
Glutamine	−3.5
Aspartic acid	−3.5
Asparagine	−3.5
Lysine	−3.9
Arginine	−4.5

*From Kyte, J., and Doolittle, R., 1982. A simple method for displaying the hydropathic character of a protein. *Journal of Molecular Biology* 157:105–132.



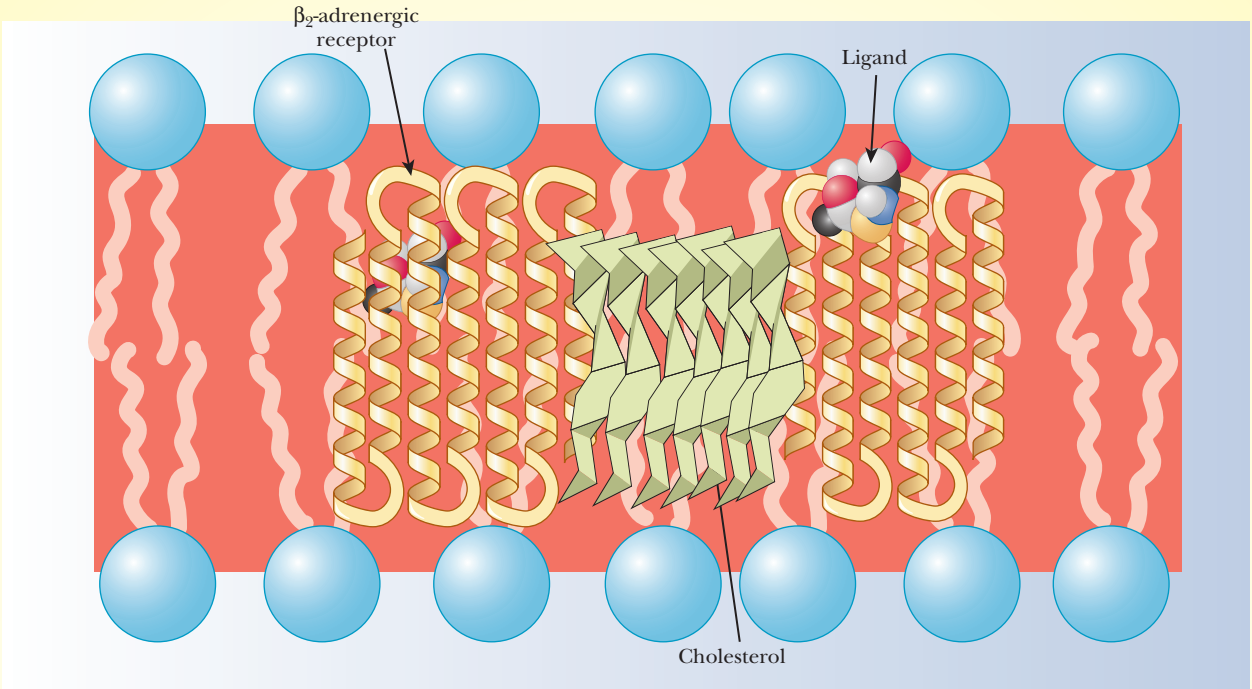
■ **FIGURE 4 Structure of rhodopsin in a membrane environment.** (a) Seven transmembrane segments are predicted by the hydropathy plot. (b) Rhodopsin in a membrane. *(From GARRETT/GRISHAM,* Biochemistry, *4E. © 2009 Cengage Learning.)*

isolated from nature. This work was an important step in understanding the nature of GPCRs.

At about the same time, a group of researchers in Belgium was cloning the genes for a number of GPCRs, including β-adrenergic receptors. They used techniques similar to those used for the α adrenergic receptor in the other work. They were able to clone genes for the β_1-, β_2-, and α_2-adrenergic receptors. They also discovered four new GPCRs. All these proteins had significant sequence homology, another important piece of information about this class of receptors.

The determination of the structure of an engineered human β_2-adrenergic receptor by x-ray crystallography was an important milestone in the understanding of these proteins. Since its discovery in 1912, x-ray crystallography has been one of the most powerful methods available to determine molecular structure. Its discoverers, the German scientist Max von Laue and the British father-son team William Bragg and William Lawrence Bragg used it to determine the crystal structure of sodium chloride and potassium chloride, a feat for which they received the 1915 Nobel Prize in physics. In that year, William Lawrence Bragg was 25 years old and serving in the British army in World War I. He was the youngest person ever to receive the Nobel Prize. Within 50 years, it was possible to use this method to determine the structure of comparatively small proteins such as myoglobin, and now determination of the structures of large and complex proteins has become a routine, but far from trivial, operation. In the case of the β_2-adrenergic receptor, detailed information about the three-dimensional structure gives insight into the details of ligand binding. A clear picture of binding can, in turn, be useful in drug design. Figure 5 shows a dimer of the receptor in the membrane (shown in yellow), with cholesterol (shown in light green) bound between the two protein molecules. The synthetic bound ligand carazolol is shown as a molecular model.

The biggest question of all is how to use the properties of GPCRs in medicine. A number of possibilities come to mind. One makes use of the fact that they

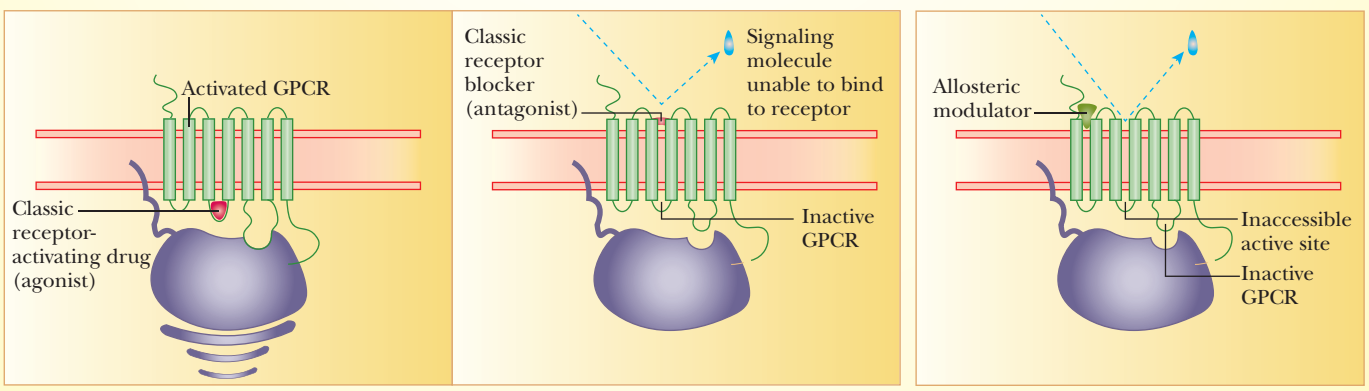


■ **FIGURE 5** Structure of the human β_2-adrenergic receptor (yellow) embedded in a lipid membrane and bound to a diffusible ligand, with cholesterol and palmitic acid (light green) between the two receptor molecules. (*Adapted from Cherezov, et al. (2007). High-resolution crystal structure of an engineered human β_2-adrenergic G protein-coupled receptor.* Science *318, 1258.*)

are allosteric proteins (see Biochemical Connections 7.1). The general mode of action of allosteric modulators is shown in Figure 6.

The main point is that the allosteric effector blocks binding of the signal molecule by changing the conformation of the GPCR, not by blocking the binding site. An important example of this effect is one connected to the treatment of AIDS. It is well known that the AIDS virus binds to helper T cells of the immune system by interacting with the cell surface protein CD4, but it has more recently come to light that a GPCR called CCR5 also plays a role in the binding. CCR5 has a binding site for gp120, the notorious viral coat protein that plays a key role in HIV infection. Allosteric modulators that bring about a conformational change in CCR5 that keep it from binding gp120 could be an important step in treatment of AIDS. Drugs based on this concept are being developed and have reached clinical trials. (People who have a genetic mutation that encodes a nonfunctional form of CCR5 tend to be highly resistant to HIV infection.) The connection between AIDS research and work on GPCRs is an example of the way in which all aspects of biochemistry are related to one another. The divisions among topics are simply a way of making it convenient for us to study this vast subject.

GPCRs play so many roles that it is possible to envision many different kinds of medications that bind to or affect this class of receptor in some way. One approach is the devise agents that will induce the cell to absorb the receptor. Some research is directed toward



■ **FIGURE 6** Comparison of the mode of action of standard drugs (binding antagonists) with drugs that operate as allosteric modulators.

finding agents that will trigger absorption of the CCR_5 protein. The AIDS virus, now matter how it mutates, cannot bind to a receptor that is no longer on the surface of the cell. Another line of research addresses the situation of a constitutive receptor, one that is active all the time, even in the absence of a signal molecule. A receptor such as this triggers uncontrolled signaling by the G protein with which it interacts. An antagonist, which inhibits the formation of the active conformation of the receptor, would not be effective when the receptor is locked in the active conformation. The goal in this case is to find an inverse agonist, a compound that will lock to protein in the inactive conformation. This line of work is rather speculative at the moment, but it does offer ideas about what advances may come as a result of basic research on these important proteins. Whatever may come, research on GPCRs is likely to lead to a number of advances in medicine. It is currently estimated that as many as 50% of medications in use now affect GPCRs.

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1 Biochemistry and the Organization of Cells

1-1 Basic Themes

How does biochemistry describe life processes?

Living organisms, such as humans, and even the individual cells of which they are composed, are enormously complex and diverse. Nevertheless, certain unifying features are common to all living things from the simplest bacterium to the human being. They all use the same types of *biomolecules,* and they all use energy. As a result, organisms can be studied via the methods of chemistry and physics. The belief in "vital forces" (forces thought to exist only in living organisms) held by 19th-century biologists has long since given way to awareness of an underlying unity throughout the natural world.

Disciplines that appear to be unrelated to biochemistry can provide answers to important biochemical questions. For example, the magnetic resonance imaging (MRI) tests that play an important role in the health sciences originated with physicists, became a vital tool for chemists, and currently play a large role in biochemical research. The field of biochemistry draws on many disciplines,

CHAPTER OUTLINE

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