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Basic Concepts of Molecular Pathology

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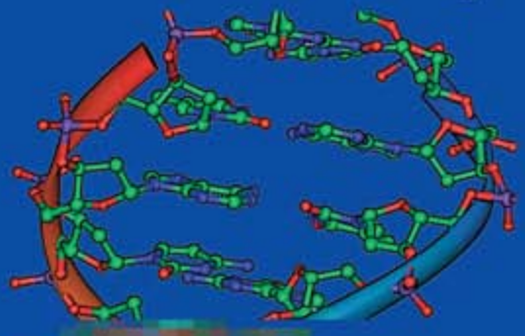

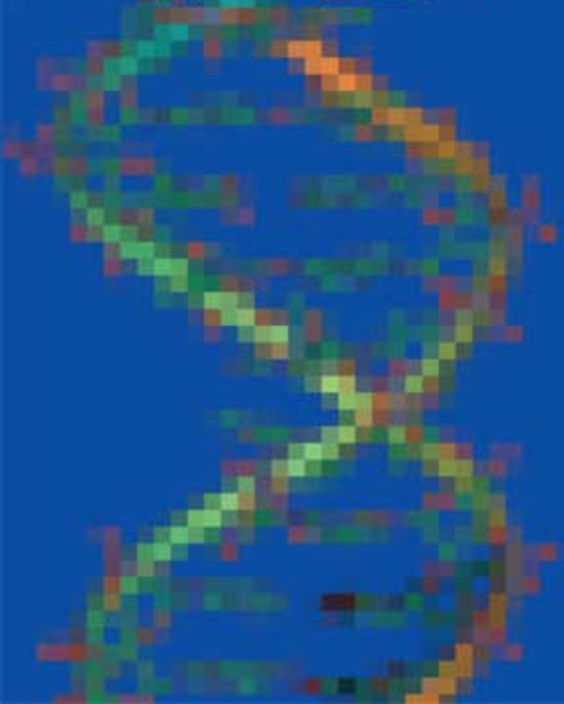

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Basic Concepts of Molecular Pathology

Edited by

Philip T. Cagle, MD
Weill Medical College of Cornell University, New York
The Methodist Hospital, Houston, Texas

and

Timothy Craig Allen, MD, JD
University of Texas Health Science Center at Tyler
Tyler, Texas

Springer

Editors
Philip T. Cagle, MD
Pathology and Laboratory Medicine
Weill Medical College of Cornell University
New York, NY
The Methodist Hospital
Houston, Texas

Timothy Craig Allen, MD, JD
Department of Pathology
University of Texas Health Science
Center at Tyler
Tyler, TX

Series editor
Philip T. Cagle, MD
Pathology and Laboratory Medicine
Weill Medical College of Cornell University
New York, NY
The Methodist Hospital
Houston, Texas

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To my wife, Kirsten

Philip T. Cagle

To my wife, Fran; and to Caitlin and Erin.

Timothy Craig Allen

Preface

The increasing role of molecular pathology in the daily practice of medicine, including the increasingly routine use of targeted molecular therapy in personalized patient care, has created a need for textbooks that bridge the gap between medical practice and basic molecular science. Each book in the *Molecular Pathology Library Series* by Springer Science+Business Media provides a review of current molecular pathology for a specific organ system. The purpose of *Basic Concepts of Molecular Pathology* is to provide a succinct background of molecular pathology terminology and concepts to serve as a foundation and reference for understanding the medically oriented organ-specific information in the other books in the series. As such, *Basic Concepts of Molecular Pathology* serves as a companion to books in the *Molecular Pathology Library Series* and also stands alone as a quick review of molecular pathology principles.

Philip T. Cagle, MD
Houston, TX
Timothy Craig Allen, MD, JD
Tyler, TX

Series Preface

The past two decades have seen an ever-accelerating growth in knowledge about the molecular pathology of human diseases, which received a large boost with the sequencing of the human genome in 2003. Molecular diagnostics, molecular-targeted therapy, and genetic therapy are now routine in many medical centers. The molecular field now impacts every field in medicine, whether clinical research or routine patient care. There is a great need for basic researchers to understand the potential clinical implications of their research, whereas private practice clinicians of all types (general internal medicine and internal medicine specialists, medical oncologists, radiation oncologists, surgeons, pediatricians, family practitioners), clinical investigators, pathologists, medical laboratory directors, and radiologists require a basic understanding of the fundamentals of molecular pathogenesis, diagnosis, and treatment for their patients.

Traditional textbooks in molecular biology present basic science and are not readily applicable to the medical setting. Most medical textbooks that include a mention of molecular pathology in the clinical setting are limited in scope and assume that the reader already has a working knowledge of the basic science of molecular biology. Other texts emphasize technology and testing procedures without integrating the clinical perspective. There is an urgent need for a text that fills the gap between basic science books and clinical practice.

In the *Molecular Pathology Library* series, basic science and technology are integrated with the medical perspective and clinical application. Each book in the series is divided according to neoplastic and nonneoplastic diseases for each of the organ systems traditionally associated with medical subspecialties.

Each book in the series is organized to provide (1) a succinct background of the essential terminology, concepts, and technology of molecular biology; (2) an overview of the broad application of molecular biology principles to disease; and (3) specific application of molecular pathology to the pathogenesis, diagnosis, and treatment of neoplastic and nonneoplastic diseases specific to each organ system. These broad section topics are divided into succinct chapters, averaging about 15–20 pages each, to cover a very specific disease entity. The chapters are written by established authorities on the specific topic from academic centers around the world. In one book, diverse subjects are included that the reader would have to pursue from multiple sources to have a clear understanding of the molecular pathogenesis, diagnosis, and treatment of specific diseases. Attempting to hunt for the full information from basic concept to specific applications for a disease from the varied sources is time consuming and frustrating. By providing this quick and user-friendly reference, understanding and application of this rapidly growing field are made more accessible to both expert and generalist alike.

As books that bridge the gap between basic science and clinical understanding and practice, the *Molecular Pathology Series* serves the basic scientist, the clinical researcher, and the practicing physician or other health care provider who require more understanding of the application of basic research to patient care, that is, from "bench to bedside." This series is unique and an invaluable resource to those who need to know about molecular pathology from a clinical, disease-oriented perspective. These books will be indispensable to physicians and health care providers in multiple disciplines, as already noted, to residents and fellows in these multiple disciplines as well as their teaching institutions, and to researchers who increasingly must justify the clinical implications of their research.

Philip T. Cagle, MD
Series Editor
Houston, TX

Contents

Contributors

Timothy Craig Allen
Department of Pathology, University of Texas Health Science Center at Tyler, Tyler, TX, USA

Roberto Barrios
Department of Pathology and Laboratory Medicine, Weill Medical College of Cornell University, New York, NY, USA
The Methodist Hospital, Houston, TX, USA

Gregory L. Blakey
Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Philip T. Cagle
Pathology and Laboratory Medicine, Weill Medical College of Cornell University, New York, NY, USA
The Methodist Hospital, Houston, TX, USA

Chung-Che (Jeff) Chang
Pathology and Laboratory Medicine, Weill Medical College of Cornell University, New York, NY, USA
The Methodist Hospital, Houston, TX, USA

William B. Coleman
Department of Pathology and Laboratory Medicine, UNC Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC, USA

Kumarasen Cooper
Department of Pathology, University of Vermont/Fletcher Allen Healthcare, Burlington, VT, USA

Sanja Dacic
Division of Anatomic Pathology, Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Daniel H. Farkas
Center for Molecular Medicine, Grand Rapids, MI, USA

Patrick L. Fitzgibbons
Department of Pathology, St. Jude Medical Center, Fullerton, CA, USA

Larry Fowler
Department of Pathology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Wieslaw Furmaga
Department of Pathology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Thomas J. Goodwin
Department of Biomedical Research and Operations Branch, NASA/Johnson Space Center, Houston, TX, USA

Kevin C. Halling
Molecular Cytology and Imaging Laboratory, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

Louise Hecker
Division of Pulmonary & Critical Care Medicine, University of Michigan Health System, Internal Medicine, Ann Arbor, MI, USA

Jennifer L. Hunt
Surgical Pathology, Head and Neck/Endocrine Pathology, AP Molecular Diagnostics Unit, Department of Anatomic Pathology, Cleveland Clinic, Cleveland, OH, USA

Jeffrey M. Jordan
Department of Pathology, University of Texas Medical Branch, Galveston, TX, USA

Dwight Oliver
Department of Pathology and Laboratory Medicine, University of Texas-Houston Medical School, Houston, TX, USA

Sem H. Phan
Department of Pathology, University of Michigan, Ann Arbor, MI, USA

Sharon C. Presnell
Cell and Tissue Technologies, Becton Dickinson, Research Triangle Park, NC, USA

Ashley G. Rivenbark
UNC Lineberger Comprehensive Cancer Center, Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, NC, USA

Pothana Saikumar
Department of Pathology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Munir Shahjahan
Pathology and Laboratory Medicine, The Methodist Hospital, Houston, TX, USA

Travis Solley
Department of Surgery, University of Texas Medical Branch, Galveston, TX, USA

Victor J. Thannickal
Division of Pulmonary & Critical Care Medicine, University of Michigan Health System, Internal Medicine, Ann Arbor, MI, USA

Reinhard Ullmann
Department of Human Molecular Genetics, Max Planck Institute for Molecular Genetics, Berlin, Germany

Manjeri A. Venkatachalam
Department of Pathology, University of Texas Health Science Center, San Antonio, TX, USA

Roger A. Vertrees
Department of Surgery, University of Texas Medical Branch, Galveston, TX, USA

Belinda J. Wagner
Scientific & Technical Operations, Tengion, Inc., Winston-Salem, NC, USA

Amy J. Wendel
Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

Youli Zu
Pathology and Laboratory Medicine, Weill Medical College of Cornell University, New York, NY, USA
The Methodist Hospital, Houston, TX, USA

1
Genes, Gene Products, and Transcription Factors

Philip T. Cagle

Introduction

Molecular pathology employs an ever-expanding array of special techniques to study nucleic acids, genes, gene products, receptors, signaling pathways, the cell cycle, and mutations. This chapter and the others in this section provide a quick review of basic terminology and concepts for the understanding of subsequent chapters.

Nucleic Acids, Genes, and Gene Products

Genes are the bits of information that code for the proteins which are necessary for structure and metabolic reactions in living tissues. Genes and the molecules that construct their protein products using the blueprints or genetic code in the genes are composed of nucleic acids. Nucleotides are the building blocks of the nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), the essential compounds that make up genes and transcribe the genetic code into proteins, respectively. Nucleotides are basic compounds composed of a sugar-phosphate backbone and a nitrogenous base. Nucleotides consist of two types of bases: purines and pyrimidines. In DNA, there are two purines (adenine, abbreviated as A, and guanine, abbreviated as G) and two pyrimidines (thymine, abbreviated as T, and cytosine, abbreviated as C). In RNA, there are also two purines (A and G) and two pyrimidines (uracil, abbreviated as U, replaces T and C). DNA is typically double stranded, with the nucleotide bases paired together as described below, and RNA is typically single stranded. When nucleotides are assembled together in a nucleic acid, formation of covalent phosphodiester bonds results in a free 5′-phosphate at the origin of the nucleic acid and a free 3′-hydroxyl at the end (terminus) of the nucleic acid. For this reason, the synthesis of the nucleic acid is said to occur in a 5′-to 3′-direction (see below).[1–9]

DNA is composed of nucleotides arranged sequentially in a deliberate order that encodes as genes for a matching sequential order of amino acids which will form proteins, as further discussed below. The nucleotides in genes are arranged in a double-stranded right-handed helix in the cell nucleus so that the purine A always binds with the pyrimidine T and the purine G always binds with the pyrimidine C in base pairs. As a result, the DNA in a double helix is arranged in complementary strands: the sequence of nucleotides in one strand of DNA is a "mirror image" of the nucleotide sequence in the other DNA strand. Groups of DNA base pairs are wrapped around small proteins called *histones*, forming arrangements of DNA called *nucleosomes*, allowing the DNA to fit within the cell nucleus. Genes are located on chromosomes that consist of DNA packaged with histone and nonhistone proteins. There are 23 pairs of chromosomes in the human, for a total of 46. Each gene is located at a specific site or locus on a specific pair of chromosomes. Because the chromosomes are in pairs and each chromosome has a locus for each gene, the genes occur as two copies or alleles, one copy or allele on each of the members of the chromosome pair. Chromosomes are ordinarily indistinct in the nuclear chromatin, but are discrete during mitosis or cell division.[1–14]

The genome is the entirety of the DNA sequence or chromosomes of an organism, or its "complete genetic complement." Genomics is the sequencing and study of genomes and cytogenetics is the study of chromosomes, traditionally through visualization of the karyotype or set of chromosomes of an organism. The somatic cells are diploid with a pair of each of the chromosomes and, therefore, two copies or alleles of each gene, one allele at the equivalent locus on each paired chromosome. The gametes are haploid, which means that these cells have only one set of each of the chromosomes with only one allele of each gene. During fertilization, the nuclear material of the two gametes combines, restoring the diploid number of chromosomes and alleles to the diploid number in the fertilized egg.[1–9]

The genotype is the genetic information in an individual's DNA, and the phenotype is how the genotype is manifested or expressed. Genotype and phenotype can differ. If two alleles are the same in one individual, they are said to be homozygous, and if the two alleles are different, they are

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said to be heterozygous. If a person is heterozygous for a gene, one allele may be expressed preferentially over the other allele, in which case the former allele is considered dominant and the latter allele is considered recessive. In this situation, the dominant allele codes for features that mask the features coded for by the recessive allele, so that the phenotype is different from the genotype. For example, a person is heterozygous for eye color and has an allele for brown eyes on one chromosome and an allele for blue eyes on the other member of that pair of chromosomes. That person will have brown eyes (the phenotype) because the allele for brown eyes is dominant over the allele for blue eyes. On the other hand, if the person is homozygous and has two recessive alleles for blue eyes, that person will have blue eyes.[1–9]

Single nucleotide polymorphisms, or SNPs (pronounced "snips"), are inherited, naturally occurring variations in one base between the DNA sequences in the same gene in two individuals and account for most of the genetic variation between individuals. Alleles or SNPs that are in close proximity on a chromosome are often inherited together as a haplotype. Polymorphisms are differences in DNA between individuals, and the most simple polymorphism is the SNP.[1–9]

Before cell division, new DNA must be synthesized from an existing strand of DNA, a process called *replication*. The synthesis of new DNA is a tightly controlled phase of the cell cycle (the S phase). The cell cycle is described in greater detail in Chap. 2. Before initiation of DNA replication, a prereplicative complex is constructed. This prereplicative complex is composed of the minichromosome maintenance protein complex (MCM), the origin recognition complex (ORC), and Cdc6/Cdc18. The S-phase kinases Cdc7 and Cdk (cyclin-dependent kinase) activate the prereplicative complex to yield an initiation complex at the origin with binding of Cdc45 to MCM. Replication is initiated at specific points in the DNA, referred to as *origins of replication*, and this creates a Y-shaped replication fork where the parental DNA duplex splits into two daughter DNA duplexes. As further described below, the duplex DNA is unwound with the assistance of special enzymes called topoisomerases, and then replication proteins, including DNA polymerases, bind to the unwound DNA.[15–60]

The synthesis of RNA, including messenger RNA (mRNA), from a strand of DNA (referred to as the *DNA template*) is known as *transcription* and is a fundamental step in the formation of the protein for which the DNA or gene codes. Condensed, inactive DNA at the periphery of the nucleus is called *heterochromatin*, and less-condensed DNA available for transcription is referred to as *euchromatin*, which is generally found in the central part of the nucleus.[1–9]

DNA polymerase is an enzyme that synthesizes DNA using single-stranded DNA as a substrate and requires a small segment of double-stranded DNA to initiate new DNA synthesis. RNA polymerase is an enzyme that synthesizes an RNA transcript from a DNA template during transcription. RNA polymerase first binds to a section of bases on the DNA called the *transcription initiation site* (TIS) or promoter "upstream" of the gene that is being transcribed. RNA polymerase I transcribes genes encoding for ribosomal RNAs (rRNAs), RNA polymerase II transcribes genes encoding for mRNAs (mRNAs), and RNA polymerase III transcribes genes encoding for transfer RNAs (tRNAs).[61–66]

The double-stranded helix of DNA must be unraveled and separated into single strands of DNA before it can undergo either transcription or replication. Topoisomerases are enzymes that break or "nick" a DNA strand, releasing the tension of the coiled helix and allowing the DNA to unwind. Transient DNA single-strand breaks are induced by topoisomerase I, and transient DNA double-strand breaks are induced by topoisomerase II. Once the DNA is separated into single strands, the DNA strand that serves as the template for the mRNA during transcription is referred to as *antisense*, and the complementary DNA strand, which has the identical sequence of bases as the mRNA (except that U replaces T), is referred to as *sense*.[67–78]

During transcription, base pairs are matched with the single strand of antisense DNA template to form a strand of mRNA. The resulting mRNA strand is a "mirror image" of the DNA template, except that uracil replaces thymine such that a DNA template with nucleotide sequence AGTC results in a strand of mRNA nucleotide sequence UCAG.[1–9]

A codon is a series of three base pair nucleotides in a gene that codes for a specific amino acid, and a series of base pair codons codes for a precise sequence of specific amino acids, resulting in the synthesis of a specific protein. The gene product is the final molecule, usually a protein, for which the gene codes that generates the effect of the gene. During translation, the mRNA, derived from the DNA template through transcription, is used as a template for the assembly of the protein product. The assembly of the protein product occurs in association with ribosomes, a component of which are rRNAs, and tRNAs add the amino acids to the protein under assembly. Each tRNA has a specific acceptor arm that attaches a specific amino acid. The tRNA ensures that the amino acid is added to the protein in the correct sequence based on the mRNA template, because the tRNA also has a specific anticodon that binds to the corresponding specific codon in the mRNA. The assembly of the protein product via mRNA is referred to as *gene expression*. Most gene expression is controlled at the level of transcription.[79–81]

Genes are made up of DNA segments called *exons* and *introns*. Exons are translated into the gene product, and introns are intervening DNA segments that are believed to play a regulatory role or serve as "punctuation" in the gene. As such, introns are spliced out of the sequence at the mRNA level, and the splice junction is the site between an exon and an intron where the splicing occurs.[1–9] A short tandem repeat (STR) consists of a sequence of two to five nucleotides that are repeated in tandem, frequently dozens of times,

in introns.[82] These STRs are found in microsatellite DNA, which is important in certain types of cancers such as colon cancer, although not significant in lung cancer.[83–99]

The end regions of chromosomes are composed of the nucleotide sequence TTAGGG repeated hundreds of times and are called *telomeres*. Telomere sequences are lost each time that a cell replicates until the cell loses its ability to divide as part of the aging process. Telomerase is a specialized DNA polymerase that replaces the DNA sequences at the telomeres of the chromosomes. Telomerase allows cells to divide indefinitely, a factor that can be important in cancer.[100–115]

Posttranslational Modifications of Gene Products

The specific sequence of amino acids in a protein imparts unique physicochemical properties that cause the polypeptide chain to fold into a tertiary structure which gives the protein its three-dimensional functional form. Domains are compact, spherical units of the three-dimensional tertiary structure.[1–9]

Dimerization is the binding of two proteins together. Binding of proteins to other proteins can enhance or inhibit their function. Dimers are frequently encountered, but trimers (three proteins), tetramers (four proteins), or other combinations can occur. Homodimers consist of two identical proteins bound together, and heterodimers consist of two different proteins bound together.[1–9]

Many proteins present in a cell are inert until they are activated by posttranslational modifications such as proteolytic cleavage or phosphorylation and become functional. The activation and inactivation of proteins by posttranslational modifications is essential in control of receptors, signaling pathways, transcription factors, and the cell cycle.

Phosphorylation and Acetylation

Phosphorylation is the addition of a phosphate group to a protein that is catalyzed by enzymes called *kinases*. Dephosphorylation is removal of a phosphate group from a protein that is catalyzed by enzymes called *phosphatases*. Many of the proteins in signaling pathways, including transcription factors, and the cell cycle are activated or inactivated by kinases and phosphatases. Depending on the domain that is phosphorylated, phosphorylation causes varying effects to a transcription factor. Phosphorylation can cause translocation (movement of a protein from the cytosol into the nucleus) and transactivation of genes, or inhibit binding proteins from binding to DNA.[116–120]

Acetylation is the addition of an acetyl group to a protein that is catalyzed by acetyltransferases, and deacetylation is the removal of an acetyl group from a protein that is catalyzed by deacetylases. Similar to kinases and phosphatases, acetyltransferases and deacetylases activate and inactivate proteins involved in various molecular events.[121]

Protein Degradation and Ubiquitinylation

To limit signaling proteins and remove damaged or abnormal proteins, protein degradation is necessary. Ubiquitinylation or poly ubiquitinylation (the ubiquitin-proteasome pathway) is the rapid degradation of proteins by reversible cross-linkage to a polypeptide called *ubiquitin*. Ubiquitin-activating enzyme (E1) activates ubiquitin, and the activated ubiquitin is transferred to a ubiquitin-conjugating enzyme (E2). The activated ubiquitin is transferred to the specific target protein by ubiquitin ligase (E3). Multiple ubiquitins are added to the protein, resulting in a polyubiquinated protein that is degraded by a large protease complex known as the *proteasome*. During this process, the ubiquitin is released to parti-cipate in more cycles of ubiquitinylation. Ubiquitinylation rapidly removes cell-cycle regulators and signaling proteins, including those involved in cell survival and cell death (apoptosis).[122]

Transcription Factors

Gene expression is primarily controlled at the level of transcription initiation. The transcriptional unit of DNA starts with the 5′-regulatory sequences and ends with the 3′-terminator signal of the gene. Gene-activating proteins are blocked from DNA by the tight binding of histone proteins to the DNA blocks. Histone acetyltransferases acetylate histones, which allows the gene-activating proteins to bind to the DNA. By blocking this process, histone deacetylases silence gene transcription.[123–133]

Transcription factors, also called *trans-acting factors* or *transactivators*, are proteins that bind to DNA and regulate the activity of RNA polymerase. Transcription factors affect gene expression directly by induction or activation of the gene or by reducing transcription levels, causing silencing or inhibition of the gene.[134–141]

Transcription factors that stimulate transcription or the synthesis of an RNA molecule from a DNA template are called *transcriptional activators*. In most cases, transcriptional activators have two domains: the DNA-binding domain recognizes and binds to a specific DNA sequence, and the transactivation domain interacts with the transcriptional machinery to induce transcription. Transcription factors can be categorized into families according to their DNA-binding domains; for example, zinc finger, leucine zipper, copper fist, basic helix-loop-helix, helix-turn-helix, and bZIP.[142–148]

Trans-acting DNA sequences encode for diffusible transcription factors that bind to distant cis-acting DNA regulatory sequences but may sometimes bind to other proteins which, in turn, bind to DNA or the transcription machinery. There are two categories of diffusible transcription factors

that bind to DNA. (1) General transcription factors are part of the basic transcription machinery by directly interacting with the RNA polymerase complex. Cis-acting DNA sequences that bind general transcription factors and function in all genes are called *promoters*. (2) Regulatory transcription factors activate or inactivate specific genes. Cis-acting DNA sequences that bind regulatory transcription factors to induce specific genes are called *enhancers*.[134–148]

The initiation of transcription by RNA polymerase requires general transcription factors. A cis-acting DNA regulatory sequence that contains adenine-thymidine-rich nucleotide sequences, referred to as a *TATA box*, is found in the promoters of many genes. The TATA-binding protein (TBP) and TBP-associated factors bind to form the general transcription factor TFIID. This transcription factor, combined with other general transcription factors (TFIIB, TFIIF, TFIIE, and TFIIH), initiate transcription by binding RNA polymerase II to the promoter. A transcription bubble is formed when the transcriptional preinitiation complex binds to a specific sequence of nucleotides and there is separation or melting of the double-stranded DNA in conjunction with histone acetylation. After separation from the preinitiation complex, the transcribing enzyme moves down the DNA template along the reading frame. During transcription elongation, the transcription bubble moves down the DNA template in a 5′- to 3′-direction (as noted earlier). Once transcription is terminated, the resultant mRNA is freed and processed before it is actively transported into the cytoplasm. In the cytoplasm, the mRNA enters the ribosome for translation of the protein product.[149–161]

Loops in the DNA bring enhancers into proximity of the transcription initiation sites even when they are located a distance away in sequence. This proximity allows the enhancers to interact with general transcription factors or RNA polymerase complexes at the promoter, allowing enhancers to stimulate gene transcription above the basal level.

An example of transcription factors is the Myc/Max/Mad network of transcription factors that regulate cell growth and death. The Myc family includes N-myc, c-myc, and L-myc. The Mad family includes Mad1, Mxi1, Mad3, Mad4, Mnt, and Mga. The Mad family functions in part as antagonists of the Myc family. These proteins form heterodimers that determine their effect. Myc/Max heterodimers activate transcription causing cell growth, proliferation, and death. Mad/Max heterodimers competitively inhibit the Myc/Max-induced transcription, causing differentiation, cell survival, and inhibition of growth and proliferation.[162–172]

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2
Receptors, Signaling Pathways, Cell Cycle, and DNA Damage Repair

Philip T. Cagle

Cell-Surface Receptors and Signal Transduction

Ligands are extracellular messenger molecules such as growth factors, inflammatory cytokines, and hormones that bind to specific receptors on the cell surface (i.e., growth factor receptors, cytokine receptors, and hormone receptors). Binding of the ligands to their receptors causes activation of second messengers in the cytosol and, eventually, activation of nuclear transcription factors. (Transcription factors are discussed in Chap. 1) The transcription factors then direct the transcription of a gene product as a result of the extracellular message (e.g., a growth factor may stimulate a growth factor receptor on the cell surface, causing activation of second messengers that eventually cause a transcription factor to cause transcription of a protein involved in cell growth). This cascade or activation and inactivation of protein messengers from the cell-surface receptors through proteins in the cytosol to the transcription factors in the nucleus is known as signal transduction. The series of steps that occurs during this process is called the signal transduction pathway or signaling pathway. Much of the activation and inactivation of proteins in signaling pathways occurs through reversible phosphorylation of tyrosine, serine, or threonine in the pathway proteins (see Chap. 1). Phosphorylation is accomplished by tyrosine kinases and serine/threonine kinases with phosphates donated from adenosine triphosphate (ATP) or guanosine triphosphate (GTP). Tyrosine kinases are much more common in signaling pathways than are serine/threonine kinases.[1-10] This discussion focuses on growth factor receptors and cytokine receptors and their associated signaling pathways.

Growth factor receptors are a common type of cell-surface receptor. Polypeptide growth factors such as epidermal growth factor (EGF) serve as ligands that bind to cell-surface receptor protein-tyrosine kinases, which causes activation of the receptor by dimerization, resulting in autophosphorylation. The activated receptor binds other proteins within the cell, leading to their phosphorylation and activation of their enzyme activity as part of the signaling pathway. The type I growth factor receptor tyrosine kinase family consists of epidermal growth factor receptor (EGFR), and ErbB1, ErbB2, ErbB3, and ErbB4 make up the type I growth factor receptor-tyrosine kinase family. In addition to EGF, EGFR has multiple ligands, including transforming growth factor-α (TGF-α).[11-16]

Several signaling pathways are well studied and important to human disease. Signaling pathways transmit the "message" from extracellular ligands such as growth factors, cytokines, and steroid hormones. Signaling pathways are involved in regulation of cell proliferation, cell differentiation, cell death or apoptosis, and cell survival. Some of the more noteworthy and established pathways are briefly reviewed. Most signaling pathways have multiple complex interactions and "crosstalk" with other pathways, so discussion of specific pathways is limited here to an abbreviated overview.

The mitogen-activated protein kinase (MAPK) family is involved in multiple signaling pathways influencing cell growth, differentiation, and apoptosis, including the Ras/Raf-1/MAPK pathway mentioned later. The MAPK family includes the extracellular signal-regulated kinases (ERK1 and ERK2); the c-Jun NH2-terminal kinases (JNK1, JNK2, and JNK3); and p38 (p38 MAP kinases α, β, and δ). The MAP kinase kinase kinases (MKKK) are activated by a wide range of agents, including growth factors, oxidative stress, inflammatory cytokines, and ultraviolet radiation. Activated MKKK activate the MAP kinase kinases (MKK), which subsequently activate the MAP kinases. Examples of MKKK include Raf-1, TGF-β-activated kinase (TAK), apoptosis signal-regulating kinase 1 (ASK1), MAP/ERK kinase kinases (MEKK), germinal center kinase (GCK), and p21-activated kinase (PAK). The ERKs have about 160 substrates and are antiapoptotic and involved in cell proliferation, cellular differentiation, and cell-cycle progression. On the other hand,

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