PEARSON CLINICAL LABORATORY SCIENCE SERIES

Clinical Laboratory Hematology

Third Edition

McKenzie | Williams

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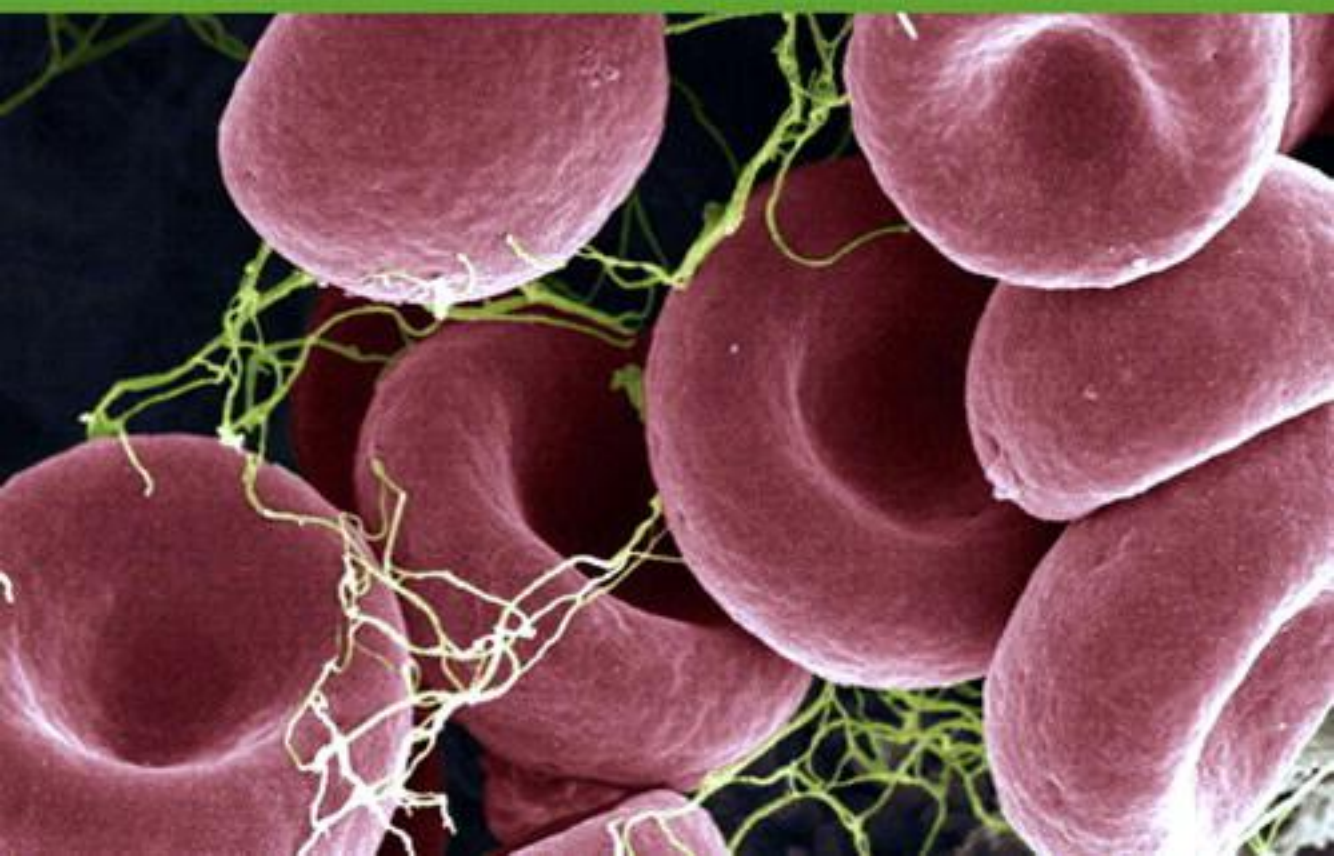

Data for reference values in these tables was compiled from multiple sources. These values will vary slightly among laboratories. Laboratories should derive reference intervals for their population and geographic location.

★ **TABLE A** Hematology Reference Values in Adults and Children (Hb, Hct, and RBC shown in conventional units; SI units in parentheses)

Age	Hb g/dL (g/L)	Hct % (L/L)	RBC × 10^6/mcL (μL) ($\times 10^{12}$/L)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Reticulocytes % ($\times 10^9$/L)
Adult							
Male	14–17.4 (140–174)	42–52 (0.42–0.52)	4.5–5.5 (4.5–5.5)	80–100	28–34	32–36	0.5–2.0 (25–75)
Female	12.0–16.0 (120–160)	36–46 (0.36–0.46)	4.0–5.0 (4.0–5.0)	80–100	28–34	32–36	0.5–2.0 (25–75)
Critical low limit	**6.6 g/dL, 1.7 SD**	**18%, 5 SD**					
Critical high limit	**19.9 g/dL, 2.7 SD**	**61%, 6 SD**					
Birth	135–200	0.42–0.60	3.9–5.9	98–123	31–37	30–36	1.7–7.0 (220–420)
2 weeks	130–200	0.39–0.65	3.6–5.9	88–123	30–37	28–35	1.0–3.0 (45–135)
							(same up to 1 year)
1 month	11–17 (110–170)	33–55 (0.33–0.55)	3.3–5.3	91–112	29–36	28–36	
2 months	9–13 (90–130)	28–42 (0.28–0.42)	3.1–4.3	84–106	27–34	28–35	
4 months	10–13 (100–130)	32–44 (0.32–0.44)	3.5–5.1	76–97	25–32	29–37	
6 months	11–14 (110–140)	31–41 (0.31–0.41)	3.9–5.5	68–85	24–30	33–37	
9 months	11–14 (110–140)	32–40 (0.32–0.40)	4.0–5.3	70–85	25–30	32–37	
1 year	13–14 (130–140)	33–41 (0.33–0.41)	4.1–5.3	71–84	24–30	32–37	
2–6 years	11.5–13.5 (115–135)	34–41 (0.34–0.41)	3.9–5.3	75–87	24–30	31–37	
6–12 years	11.5–15.5 (115–155)	35–45 (0.35–0.45)	4.0–5.2	77–95	25–33	31–37	

Reference intervals derived from combined data. Critical limits are the low and high boundaries of life-threatening values. Results that fall below the low critical limit and above the high critical limit are "panic values" or critical results that require emergency notification of physicians. These limits were derived by Dr. George Kost from a national survey of 92 institutions.

Source: Data from article by Kost GJ: Critical limits for urgent clinician notification at US Medical Centers. *JAMA*. 1990; 263:704.

★ TABLE B Age and Race-Specific Reference Intervals for Leukocyte Count and Differential[a]

	Birth	6 Months	4 Years	Adult	Adult of African Descent
Total leukocyte count ($\times 10^9$/L)	9.0–30.0	6.0–18.0	4.5–13.5	4.5–11.0	3.0–9.0
Segmented neutrophil: percent (%)	50–60	25–35	35–45	40–80	45–55
Absolute ($\times 10^9$/L)	4.5–18.0	1.5–6.3	1.5–8.5	1.8–7.0	1.5–5.0
Band neutrophil percent (%)	5–14	0–5	0–5	0–5	0–5
Absolute ($\times 10^9$/L)	0.5–4.2	0–1.0	0–0.7	0–0.7	0–0.7
Lymphocyte percent (%)	25–35	55–65	50–65	25–35	35–45
Absolute ($\times 10^9$/L)	2.0–11.0	4.0–13.5	2.0–8.8	1.0–4.8	1.0–4.8
Monocyte percent (%)	2–10	2–10	2–10	2–10	2–10
Absolute ($\times 10^9$/L)	0.2–3.0	0.1–2.0	0.1–1.4	0.1–0.8	0.1–0.8
Eosinophil percent (%)	0–5	0–5	0–5	0–5	0–5
Absolute ($\times 10^9$/L)	0–1.5	0–0.9	0–0.7	0–0.4	0–0.4
Basophil percent (%)	0–1	0–1	0–1	0–1	0–1
Absolute ($\times 10^9$/L)	0–0.6	0–0.4	0–0.3	0–0.2	0–0.2

[a]Compiled from multiple sources. Values may vary among sources and laboratories.

★ TABLE C Other Hematology Reference Values

Analyte	Reference Value
Immature reticulocyte fraction (IRF)	0.09–0.31
RDW	12–14.6
Platelet count	150–400 $\times$ 10^9/L
MPV	6.8–10.2 fL
Sedimentation rate	
Male <50 years	0–15 mm/hr
>50 years	0–20 mm/hr
Female <50 years	0–20 mm/hr
>50 years	0–30 mm/hr
Zeta sedimentation rate	
Male	40–52
Female	40–52
Cerebrospinal fluid	
Erythrocytes	0
Leukocytes	<5/mcL

Clinical Laboratory Hematology

Third Edition

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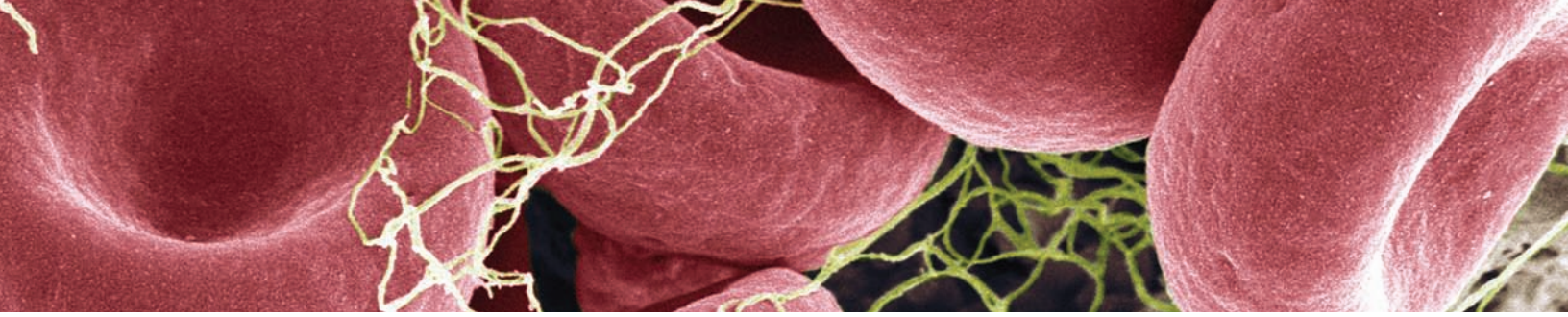
To my family, the wind beneath my wings, Gary, Scott, Shawn, Belynda, and Dora; my special grandchildren Lauren, Kristen, Weston, Waylon, and Wyatt; to the memory of my parents, George and Helen Olson.

Shirlyn B. McKenzie

For my mother, Mary Williams, who gave her children roots as well as wings; for Lee, Laurie, Roger, and Richard, who sustain my roots; for Dulaney, Corie, Chris, Ava, and Holden, whom I love as my own; and to the memory of my father, David Williams.

J. Lynne Williams



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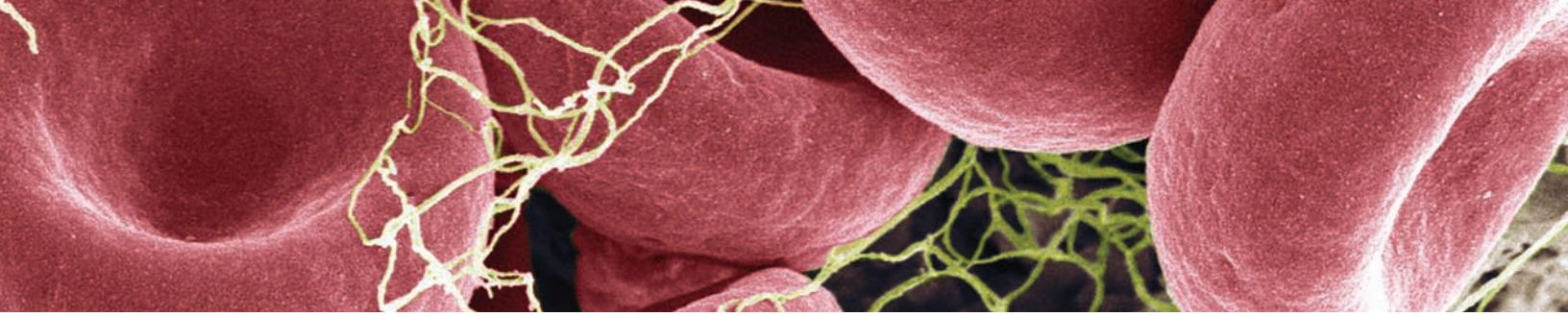

Foreword

Clinical Laboratory Hematology is part of Pearson's Clinical Laboratory Science (CLS) series of textbooks, which is designed to balance theory and practical applications in a way that is engaging and useful to students. The authors of and contributors to *Clinical Laboratory Hematology* present highly detailed technical information and real-life case studies that will help learners envision themselves as members of the health care team, providing the laboratory services specific to hematology that assist in patient care. The mixture of theoretical and practical information relating to hematology provided in this text allows learners to analyze and synthesize this information and, ultimately, to answer questions and solve problems and cases. Additional applications and instructional resources are available at www.pearsonhighered.com/healthprofesionsresources.

We hope that this book, as well as the entire series, proves to be a valuable educational resource.

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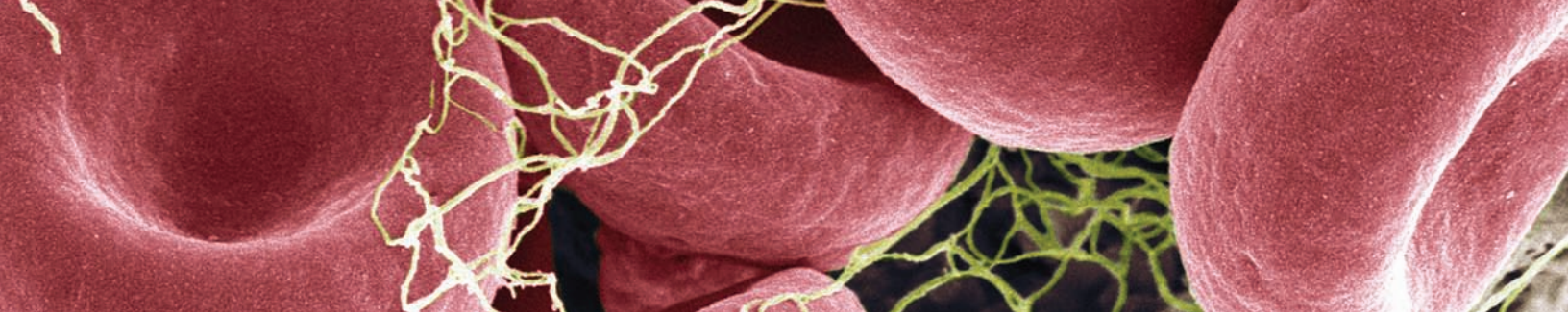


Preface

As with the first two editions, the third edition of *Clinical Laboratory Hematology* is designed to be a comprehensive resource that medical laboratory technician (MLT) and medical laboratory science (MLS) students can use in all their hematology courses. Laboratory practitioners will find the book a welcome resource to help them keep up with advances in the field. The book also is suited for use by students in other health care professions including pathology, medicine, physician assistant, and nursing. This edition is thoroughly updated to include the latest in advances in laboratory medicine. Each chapter has a similar format; the striking visual design makes it easy for readers to find information on each topic. Multiple supplemental learning tools for students and teaching resources for the instructor, including a website with resources available by chapter, are available. In summary, the book is not just a book but a package of learning tools.

ORGANIZATION

We believe that students must have a thorough knowledge of normal hematopoiesis and cell processes to understand the pathophysiology of hematologic/hemostatic diseases, evaluate and correlate laboratory test results, and ensure the appropriate utilization of the laboratory in diagnosis and patient follow-up. Thus, this book is organized so that the first 10 chapters give the students a comprehensive base of knowledge about blood cell proliferation, maturation, and differentiation and the processes that control hematopoiesis. Section One (Chapters 1–2) includes an introduction to hematology and hematopoiesis, including cell morphology and the cell cycle and its regulation. This introduction includes a description of cellular processes at the molecular level, which could be new material for some students and a basic review for others. The reader might want to review these chapters before beginning a study of neoplastic disorders. Section Two (Chapters 3–10) includes chapters on normal hematopoiesis, including a description of the structure and function of hematopoietic tissue and organs, erythropoiesis, leukopoiesis, and hemoglobin. In this third edition, the chapter on leukocytes is divided into two separate chapters: granulocytes/monocytes (Chapter 7) and lymphocytes (Chapter 8). An introductory chapter on platelets (Chapter 9) was added to this section to complete the discussion of normal blood cells. Details of platelet function and physiology are found in Section Eight, "Hemostasis." Chapter 10, "The Complete Blood Count and Peripheral Blood Smear Examination" is a new chapter that describes the information that can be gained about blood cells from these frequently ordered laboratory tests. Most of the remaining chapters refer to the tests that are described in this chapter.

The next three sections include discussions of hematologic disorders. Section Three (Chapters 11–20) begins with an introduction to anemia (Chapter 11). In this edition, we combined the introduction to anemia and the introduction to hemolytic anemia into one chapter because many anemias have a hemolytic component. This chapter is followed by chapters on the various anemias. Each anemia is discussed in the following manner: introduction, etiology, pathophysiology, clinical findings, laboratory findings, and therapy. This format helps readers understand what laboratory tests can help in diagnosis and how to interpret the results of these tests. Section Four (Chapters 21 and 22) covers the nonmalignant disorders of leukocytes. Section Five (Chapters 23–29) is a study of hematopoietic neoplasms. This section begins with an overview of these disorders to help students understand the classification, terminology, and pathophysiology of neoplasms and the laboratory's role in diagnosis and therapy. As a part of this section, we included a chapter on stem cell therapy (Chapter 29) because it is a frequently used therapy for these neoplasms and the laboratory plays a critical role in harvesting the stem cells and preparing them for transplant. Molecular studies are becoming a major diagnostic tool for neoplastic disorders and are discussed within each chapter as well as in the chapter devoted to molecular diagnostics (Chapter 42). Some instructors might prefer to cover Section Eight, the study of bone marrow (Chapter 38), flow cytometry (Chapter 40), cytogenetics (Chapter 41), and molecular diagnostics (Chapter 42) before teaching Section Five or integrate this material with Section Five. Some hematology courses do not include these topics, or instructors might not want to cover them in the depth presented in this book.

Section Six (Chapter 30) is a study of body fluids from a hematologic perspective and thus includes a large number of photographs of cells found in body fluids. This chapter has been reorganized and revised extensively to give a more complete perspective on body fluid analysis. Discussions of semen analysis and amniotic fluid lamellar body counts have been added. Additional photographs have been added to the online resources. Not all hematology courses include

this topic, but the chapter is written in such a way that it can be used separately in a body fluid course.

Section Seven (Chapters 31–36) is a study of hemostasis. Chapters on normal hemostasis include primary and secondary hemostasis and fibrinolysis. They are followed by three chapters on disorders of hemostasis. Chapter 36 describes the testing procedures for hemostasis, including information on automation. This chapter has been revised by laboratory coagulation specialists and describes an extensive collection of coagulation procedures; additional detailed information on hemostasis testing is available on the chapter's website. These procedures can be downloaded and used as is or adapted for use in student laboratories.

Section Eight (Chapters 37–42) includes chapters on test procedures that help in the diagnosis of hematologic disorders. Automation in hematology is included in Chapter 39. Extensive additional information is included on the book's website and includes step-by-step procedures for some tests, graphs, tables, figures, and printouts of abnormal results using various hematology analyzers. Chapter 42 is designed to introduce molecular procedures and their use in detecting various hematologic and hemostatic disorders. A background in genetics is suggested before students begin this chapter.

Section Nine (Chapter 43) is a thorough discussion of quality assessment in the hematology laboratory. Problems discussed include common abnormal results, errors, and alert flags. Corrective action to take to resolve these problems is described. Several excellent tables help to quickly find needed information. We suggest that these tables be read early in the course of study because they can be used periodically when attempting to interpret and correlate laboratory test results. Chapter 10 refers the reader to these tables because it discusses interpretation of test results and abnormalities in the CBC.

The text emphasizes the effective, efficient, and ethical use of laboratory tests. The clinical laboratory professional is in an ideal position to assist physicians in interpreting laboratory test results and choosing the best reflex tests to arrive at a diagnosis or evaluate therapy. Many laboratories develop algorithms to assist in these tasks. This text includes several algorithms that some laboratories use. To save page space in the text, some algorithms are on the website.

SUITABLE FOR ALL LEVELS OF LEARNING

The book is designed for both MLT and MLS students. Using only one textbook for both levels is beneficial and economic for laboratory science programs that offer both levels of instruction. It also is helpful for programs that have developed articulated MLT to MLS curricula. The MLS program can be confident of the MLT's knowledge in hematology without doing a time-consuming analysis of the MLT course.

Objectives are divided into two levels: Level I (basic) and Level II (advanced). MLT instructors who reviewed the objectives for this text generally agreed that most Level I objectives are appropriate for the MLT body of knowledge. They also indicated that some Level II objectives are appropriate for MLTs. MLS students should be able to meet both Level I and Level II objectives in most cases. If the MLS program has two levels of hematology courses—Level I and Level II—this book can be used for both.

All instructors, regardless of discipline or level, need to communicate to their students what is expected of them. They might want their students to find the information in the text that allows them to satisfy selected objectives, or they might assign particular sections to read. If not assigned specific sections to read, the MLT students may read more than expected, which is not a bad thing! The two levels of review questions at the end of each chapter are matched to the two levels of objectives.

The Case Study questions and the Checkpoints are not delineated by level. All students should try to answer as many of them as possible to assess their understanding of the material.

We recognize that there are many approaches to organizing a hematology course and that not all instructors teach in the same topic sequence or at the same depth. Thus, we encourage instructors to use the book by selecting appropriate chapters and objectives for their students based on their course goals. Each program should assess what content fits its particular curriculum. The layout of the book is such that instructors can select the sequence of chapters in an order that fits their course design, which might not necessarily be the sequence in the book. However, we recommend that the course begin with Sections One and Two and that the chapters "Introduction to Anemia" and "Introduction to Hematopoietic Neoplasms" be studied before the individual chapters that follow on these topics. The Background Basics sections help the instructor determine which concepts students should master before beginning each chapter. This feature helps instructors customize their courses. Some hematology courses might not include some chapters on subjects such as molecular techniques, cytogenetics, flow cytometry, and body fluids but they might be helpful in other courses.

As a note, this text uses mc as an abbreviation for micro, which replaces μ. Thus abbreviations of mcg, mcL, mcM replace those that use the Greek letter "mu" (μg, μL, μM).

UNIQUE PEDAGOGICAL FEATURES

The text has a number of unique pedagogical features to help the students assimilate, organize, and understand the information. Each chapter begins with a group of components intended to set the stage for the content to follow.

- The **Objectives** comprise two levels: Level I for basic or essential information and Level II for more advanced information. Each instructor must decide what to expect their students to know.
- The **Key Terms** feature alerts students to important terms used in the chapter and found in the glossary.
- The **Background Basics** component alerts students to material that they should have learned or reviewed before starting the chapter. In most cases, these features refer readers to previous chapters to help them find the material if they want to review it.
- The **Overview** gives readers an idea of the chapter content and organization.

- The **Case Study** is a running case feature that first appears at the beginning of a chapter and focuses the students' attention on the subject matter that the chapter covers.
- Appropriate places throughout the chapter provide additional information on the case, such as additional laboratory test results followed by questions that relate to the material presented in preceding sections. The book's website provides the answers to Case Study questions.
- The **Checkpoints** components are integrated throughout the chapter. They are questions that require students to pause along the way to recall or apply information covered in preceding sections. The answers are provided on the book's website.
- A **Summary** concludes the text portion of each chapter to help students bring all the material together.
- **Review Questions** appear at the end of each chapter. The two sets of questions, Level I and Level II, are referenced and organized to correspond to the Level I and Level II objectives. Answers are provided in the Appendix.

The page design features a number of enhancements intended to aid the learning process.

- **Colorful symbols** are used to identify callouts for tables (★) and figures (■) within the chapter text to help students quickly cross-reference from the tables and figures to the text.
- **Figures and tables** are used liberally to help students organize and conceptualize information. This is especially important for visual learners.
- **Microphotographs** are displayed liberally in the book and are typical of those found in a particular disease or disorder. Students should be aware that cell variations occur and that blood and bone marrow findings do not always mimic those found in textbooks. The legend for each microphotograph gives the original magnification but sometimes the image was zoomed to enhance detail.

WHAT'S NEW

Major changes in the text organization are listed here as a quick reference for instructors. In addition to updating, the following changes have been made:

- The leukocyte chapter has been split into two chapters (7 and 8). Chapter 7 includes granulocytes and monocytes; chapter 8 includes lymphocytes.
- An introductory chapter on platelets (Chapter 9) was added to complete the section on blood cells. More detailed information is included in Section Seven, Hemostasis.
- A chapter was added (Chapter 10, The Complete Blood Count and Peripheral Blood Smear Evaluation) to introduce the student to the results and interpretation of two of the most common laboratory tests in hematology.
- Section Five, Hematopoietic Neoplasms, is thoroughly updated using the WHO 2008 classification.
- The body fluid chapter (Chapter 30) was expanded to include more information on procedures and additional body fluids including semen and amniotic fluid. Additional photos of cells are available on the chapter's website.
- Automation in hemostasis testing was moved to the chapter about hemostasis procedures (Chapter 36).
- Chapter 39 includes automation in the hematology laboratory.
- Appendix A contains the answers to chapter review questions. The answers to the case study questions and checkpoints are available on the website.
- Two new comprehensive tables were added to the appendices. The table in Appendix B was developed through a collaborative effort of several authors. It lists hematopoietic neoplasms with the following information on each: immunophenotype using CD markers, cytogenetic abnormalities, and genotypic findings. This table provides a ready reference for information from the chapters in Section Five (Neoplastic Hematologic Disorders) and Section 8 (Hematology Procedures). The table in Appendix C is a comprehensive classification of hematopoietic, lymphopoietic, and histiocytic/dendritic neoplasms using the 2008 WHO classification system.

A COMPLETE TEACHING AND LEARNING PACKAGE

A variety of ancillary materials designed to help instructors be more efficient and effective and students more successful complements this book.

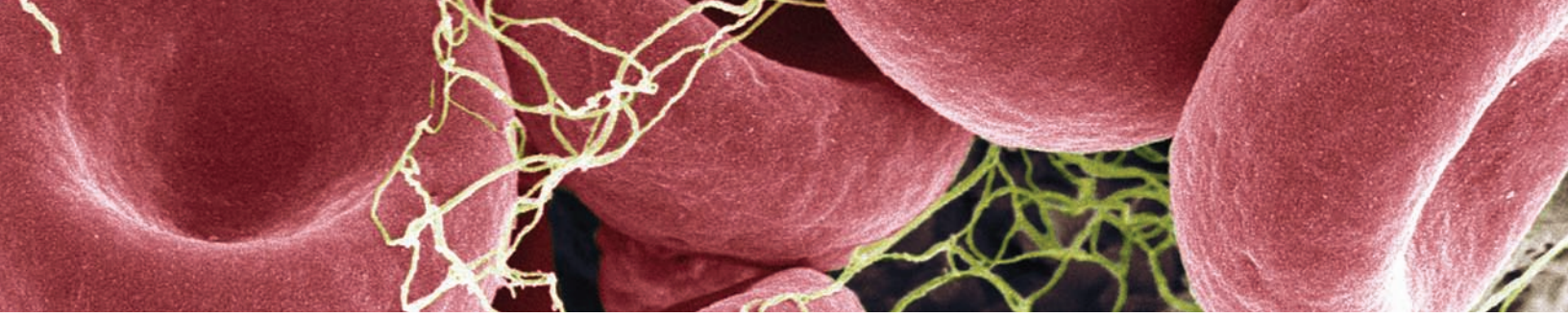
An **Instructor's Resource Center** is available upon adoption of the text and gives the instructor access to a number of powerful tools in an electronic format. The following materials are downloadable:

- The **MyTest** feature includes questions to allow instructors to design customized quizzes and exams. The MyTest guides instructors through the steps to create a simple test with drag-and-drop or point-and-click transfer. Test questions are available either manually or randomly and use online spell checking and other tools to quickly polish the test content and presentation. Instructors can save their tests in a variety of formats both local and network, print as many as 25 variations of a single test, and publish the tests in an online course.
- The **PowerPoint Lectures** tool contains key discussion points and color images for each chapter. This feature provides dynamic, fully designed, integrated lectures that are ready to use, allowing instructors to customize the materials to meet their specific course needs. These ready-made lectures will save instructors time and allow an easy transition into using *Clinical Laboratory Hematology*.
- The **Image Library** feature contains all of the images from the text. Instructors have permission to copy and paste these images into PowerPoint lectures, printed documents, or website as long as they are using *Clinical Laboratory Hematology* as their course textbook.

- The **Instructor's Resource Manual** tool in PDF and Word formats can be accessed.
- The **Bonus Image Library** feature contains microphotographs of normal and abnormal blood cells filed by chapter. These can be downloaded into instructors' digital presentations or used on password-protected course websites.

COMPANION RESOURCES (WWW.PEARSONHIGHERED.COM/HEALTHPROFESSIONSRESOURCES)

This online resource page is completely unique to the market. The website presents additional figures, tables, and information for readers. For procedure chapters, the website includes detailed laboratory procedures that can be adapted and printed for use in the laboratory.



Acknowledgments

Writing a textbook is a complicated task that requires a team of dedicated authors, editors, copy editors, artists, permission researchers, educators, practitioners, content reviewers, project and program managers, and many other individuals behind the scenes. The team that Pearson and the editors put together to make the third edition of this book an excellent hematology and hemostasis resource for students and health care practitioners worked tirelessly over several years to bring the project to completion. The new and returning authors ensured that their chapters were up to date and accurate. Content reviewers and users of the second edition provided helpful suggestions that were incorporated into the chapters. Dr. Brooke Solberg had an important role in reviewing the body fluid chapter and making recommendations that enhanced the chapter's content and organization. We offer our thanks to this group who ensured a quality textbook for a wide audience.

Andrea Klingler was our daily contact who kept us on track even though it meant multiple deadline revisions. She was understanding when our mistakes meant more work for her. Her gentle prodding was evident and appreciated. Her editing was superb.

Rebecca Lazure came into the picture later in the process and played an important role in final copyediting. Patty Gutierrez was instrumental in working with permission researchers to obtain permission for use of copyright works.

John Goucher started the ball moving on the third edition. He had faith in us and provided support and encouragement for another edition of *Clinical Laboratory Hematology*. Jonathan Cheung and Nicole Rangonese were essential in finding authors for support materials including PowerPoints, test questions, and the instructor's guide. This group of author educators contributed behind the scenes to enhance the instructors' use of this book.

A very special thanks goes to Dr. Kristin Landis-Piwowar, Consulting Editor, who accepted a critical editing role late in our process. Her knowledge and expertise in molecular diagnostics proved invaluable. Her attention to detail, writing ability, and suggestions for organization are evident in her editing. Most notably, she was always willing and able to take additional tasks to help keep us on track.

Although he wasn't involved in producing this edition, Mark Cohen was responsible for the creation of the first edition of this text. His keen insights into developing a unique textbook design with pedagogical enhancements has helped *Clinical Laboratory Hematology* become a leading textbook in the field of clinical laboratory science.

Thank you, Pearson, for having faith in us to publish a third edition. Thank you for providing the special team of experts to help us accomplish this task. We recognize that the job is not over but will require the efforts of sales and marketing to ensure widespread use and adoption.

SBM and JLW

The reason I took the task of writing my first hematology textbook was that as an instructor for medical laboratory science students, I could not find a suitable text for them. Thus, my former students were the inspiration for this book. Thank you for your feedback to help make each edition better.

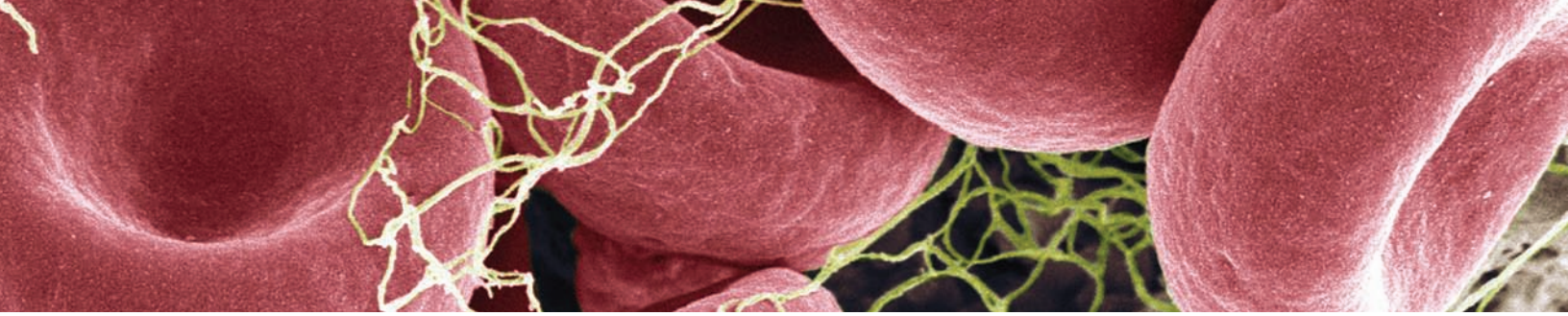
Writing and editing a text of this size is a monumental job. I am privileged to work with my brilliant fellow coeditor and friend, Dr. J. Lynne Williams, who spent many hours of research on topics before editing to ensure that the chapters are up to date and accurate. Her ability to recognize errors is without equal. She spent many late hours at the office to complete editing tasks. We have similar philosophies about teaching hematology and often discussed how to best present the information in this book.

During the time this book was under development, my professional life took over many hours of my personal life. Many thanks to my husband and best friend for his support, sacrifices, and understanding during some very stressful times so this book could become a reality. My parents, George and Helen Olson, instilled in me the confidence that I could accomplish anything I set my heart to. This mind-set has stuck with me through life, especially in this task. I hope that through example I have provided the same to my children and grandchildren.

SBM

I extend a special thank you to my colleagues in the Biomedical Diagnostic and Therapeutic Sciences program at Oakland University—Dr. Kristin Landis-Piwowar, Dr. Sumit Dinda, Lisa DeCeuninck, and our many part-time instructors—who kept the programs moving forward while I was working on this new edition—and to the BDTS students of the past 2 years who tolerated a distracted and often absent-minded professor. To all my former students: You have been my inspiration to try to create a meaningful and useful book to support your educational endeavors. But especially to my coeditor, Dr. Shirlyn McKenzie: thank you for the privilege of accompanying you on this wonderful journey.

JLW



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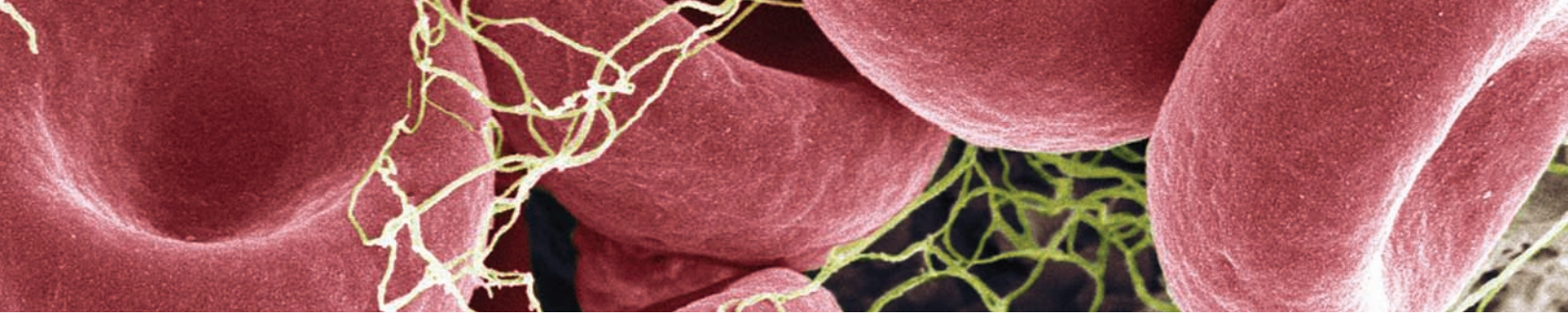

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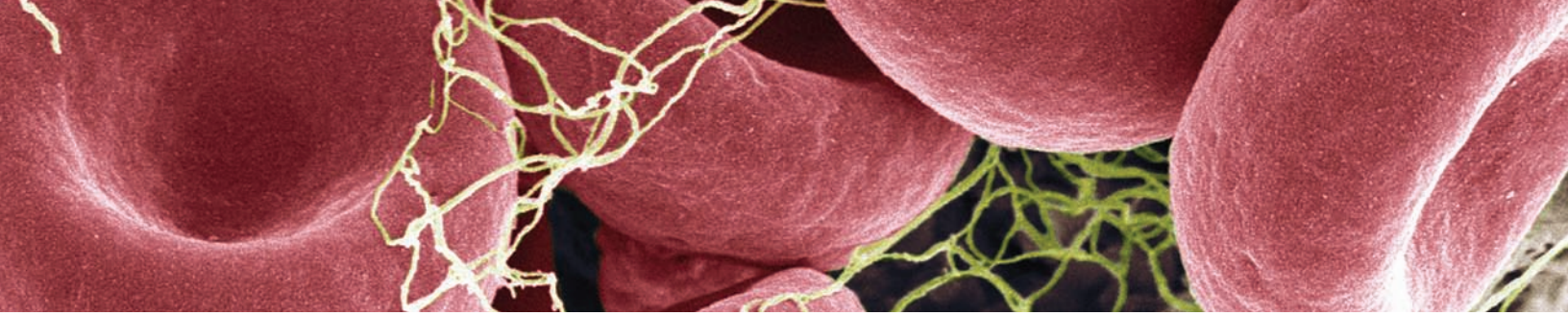
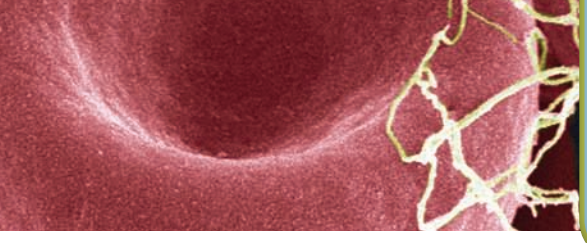


Select Abbreviations Used

AHG	Antihuman globulin
AIDS	Acquired immune deficiency syndrome
AIHA	Autoimmune hemolytic anemia
AL	Acute leukemia
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
ANLL	Acute nonlymphocytic leukemia
APTT	Activated partial thromboplastin time
ARC	AIDS-related complex
Band	Nonsegmented neutrophil
BCR	B-cell receptor
BT	Bleeding time
CBC	Complete blood count
CD	Cluster of differentiation
CDK	Cyclin-dependent kinase
cDNA	Complementary DNA
CEL, NOS	Chronic eosinophilic leukemia, not otherwise specified
CFU	Colony-forming unit
CGL	Chronic granulocytic leukemia
CHr	Reticulocyte hemoglobin
CHCMr	Mean corpuscular hemoglobin concentration of the reticulocyte
CKI	Cyclin-dependent kinase inhibitor
CLL	Chronic lymphocytic leukemia
CLP	Common lymphoid progenitor
CML	Chronic myeloid (myelogenous) leukemia
CMML	Chronic myelomonocytic leukemia
CMV	Cytomegalovirus
CNL	Chronic neutrophilic leukemia
DAF	Decay-accelerating factor
DAT	Direct antiglobulin test
DIC	Disseminated intravascular coagulation
dL	Deciliter
DNA	Deoxyribonucleic acid
DVT	Deep vein thrombosis
EBV	Epstein-Barr virus
EPO	Erythropoietin
ER	Endoplasmic reticulum
ET	Essential thrombocythemia
FA	Fanconi's anemia
FAB	French-American-British
FFP	Fresh frozen plasma
G6PD	Glucose-6-phosphate dehydrogenase
GMP	Granulocyte/monocytes progenitor
Hb	Hemoglobin
Hct	Hematocrit
HDFN	Hemolytic disease of the fetus and newborn
HES	Hypereosinophilic syndrome
HPFH	Hereditary persistence of fetal hemoglobin
HUS	Hemolytic uremic syndrome
IAT	Indirect antiglobulin test
Ig	Immunoglobulin
INR	International normalized ratio
IRF	Immature reticulocyte fraction
ISC	Irreversibly sickled cell
ISI	International sensitivity index
ITP	Immune thrombocytopenia also called Idiopathic thrombocytopenic purpura
L	Liter
LAP	Leukocyte alkaline phosphatase
LCAT	Lecithin-cholesterol acyl transferase
LD	Lactic dehydrogenase
Lymph	Lymphocyte
MAHA	Microangiopathic hemolytic anemia
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular (cell) volume
MDS	Myelodysplastic syndrome
MEP	megakaryocytic/erythroid progenitor
MHC	Major histocompatibility complex
mcG	Microgram
mcL	Microliter
mcM	Micrometer
mL	Milliliter
Mono	Monocyte
MPD	Myeloproliferative disorder
MPN	Myeloproliferative neoplasm
MW	Molecular weight
NRBC	Nucleated red blood cell
PAS	Periodic acid-Schiff

PCH	Paroxysmal cold hemoglobinuria
PCR	Polymerase chain reaction
PDW	Platelet distribution width
PIVKA	Protein induced by vitamin-K absence (or antagonist)
PK	Pyruvate kinase
PMN	Polymorphonuclear neutrophil
PNH	Paroxysmal nocturnal hemoglobinuria
PT	Prothrombin time
RA	Refractory anemia
RB	Retinoblastoma
RAEB	Refractory anemia with excess blasts
RARS	Refractory anemia with ring sideroblasts
RBC	Red blood cell
RDW	Red cell distribution width
RER	Rough endoplasmic reticulum
RET-He	Reticulocyte hemoglobin content measured by Sysmex instrument
rHuEPO	Recombinant human erythropoietin
RNA	Ribonucleic acid
RPI	Reticulocyte production index
SCIDS	Severe combined immunodeficiency syndrome
Seg	Segmented neutrophil
SER	Smooth endoplasmic reticulum
SLL	Small lymphocytic lymphoma
TCR	T-cell receptor
TF	Transcription Factor
TIBC	Total iron-binding capacity
TPO	Thrombopoietin
TRAP	Tartrate-resistant acid phosphatase
TTP	Thrombotic thrombocytopenic purpura
UTR	Untranslated region
VWF	von Willebrand factor
WBC	White blood cell
WHO	World Health Organization



1 Introduction

SHIRLYN B. McKENZIE, PhD

Objectives—Level I and Level II

At the end of this unit of study, the student should be able to:

1. Compare the reference intervals for hemoglobin, hematocrit, erythrocytes, and leukocytes in infants, children, and adults.
2. Identify the function of erythrocytes, leukocytes, and platelets.
3. Describe the composition of blood.
4. Explain the causes of change in the steady state of blood components.
5. Describe reflex testing, and identify the laboratory's role in designing reflex testing protocols.
6. Define hemostasis and describe the result of an upset in the hemostatic process.
7. Identify hematology and hemostasis screening tests.
8. List the three components of laboratory testing and correlate errors with each component.

Key Terms

Activated partial thromboplastin time (APTT)
Complete blood count (CBC)
Diapedese
Erythrocyte
Hematocrit
Hematology
Hematopoiesis
Hemoglobin
Hemostasis
Leukocyte
Plasma
Platelet
Prothrombin time (PT)
RBC index
Red blood cell (RBC)
Reflex testing
Thrombocyte
White blood cell (WBC)

Background Basics

Students should complete courses in biology and physiology before beginning this study of hematology.

Chapter Outline

CASE STUDY

We will address this case study throughout the chapter.

Aaron, a 2-year-old male, was seen by his pediatrician because he had a fever of 102 to 104°F over the past 24 hours. Aaron was lethargic. Before this, he had been in good health except for two episodes of otitis.

Consider why the pediatrician might order laboratory tests and how this child's condition might affect the composition of his blood.

OVERVIEW

Hematology is the study of blood and blood-forming organs. The hematology laboratory is one of the busiest areas of the clinical laboratory. Even small, limited-service laboratories usually offer hematology tests. This chapter is an introduction to the composition of blood and the testing performed in the hematology laboratory to identify the presence and cause of disease.

INTRODUCTION

Blood has been considered the essence of life for centuries. One of the Hippocratic writings from about 400 B.C. describes the body as being a composite of four humors: black bile, blood, phlegm, and yellow bile. It is thought that the theory of the four humors came from the observation that four distinct layers form as blood clots in vitro: a dark-red, almost black, jellylike clot (black bile); a thin layer of oxygenated red cells (blood); a layer of white cells and platelets (phlegm); and a layer of yellowish serum (yellow bile).[1] Health and disease were thought to occur as a result of an upset in the equilibrium of these humors.

The cellular composition of blood was not recognized until the invention of the microscope. With the help of a crude magnifying device that consisted of a biconvex lens, Leeuwenhoek (1632–1723) accurately described and measured the **red blood cells** (also known as **RBCs** or **erythrocytes**). The discovery of **white blood cells** (also known as **WBCs** or **leukocytes**) and **platelets** (also known as **thrombocytes**) followed after microscope lenses were improved.

As a supplement to these categorical observations of blood cells, Karl Vierordt in 1852 published the first quantitative results of blood cell analysis.[2] His procedures for quantification were tedious and time consuming. After several years, many others attempted to correlate blood cell counts with various disease states.

Improved methods of blood examination in the 1920s and the increased knowledge of blood physiology and blood-forming organs in the 1930s allowed anemias and other blood disorders to be studied on a rational basis. In some cases, the pathophysiology of hematopoietic disorders was realized only after the patient responded to experimental therapy.

Contrary to early hematologists, modern hematologists recognize that alterations in the components of blood are the *result* of disease, not a *primary cause* of it. Under normal conditions, the production of blood cells in the bone marrow, their release to the peripheral blood, and their survival are highly regulated to maintain a steady state of morphologically normal cells. Quantitative and qualitative hematologic abnormalities can result when an imbalance occurs in this steady state.

COMPOSITION OF BLOOD

Blood is composed of a liquid called **plasma** and of cellular elements, including leukocytes, platelets, and erythrocytes. The normal adult has about 6 liters of this vital fluid, which composes from 7% to 8% of the total body weight. Plasma makes up about 55% of the blood volume; about 45% of the volume is composed of erythrocytes, and 1% of the volume is composed of leukocytes and platelets. Variations in the quantity of these blood elements are often the first sign of disease occurring in body tissue. Changes in diseased tissue may be detected by laboratory tests that measure deviations from normal in blood constituents. Hematology is primarily the study of the formed cellular blood elements.

The principal component of plasma is water, which contains dissolved ions, proteins, carbohydrates, fats, hormones, vitamins, and enzymes. The principal ions necessary for normal cell function include calcium, sodium, potassium, chloride, magnesium, and hydrogen. The main protein constituent of plasma is albumin, which is the most important component in maintaining osmotic pressure. Albumin also acts as a carrier molecule, transporting compounds such as bilirubin and heme. Other blood proteins carry vitamins, minerals, and lipids. Immunoglobulins, synthesized by lymphocytes, and complement are specialized blood proteins involved in immune defense. The coagulation proteins responsible for **hemostasis** (arrest of bleeding) circulate in the blood as inactive enzymes until they are needed for the coagulation process. An upset in the balance of these dissolved plasma constituents can indicate a disease in other body tissues.

Blood plasma also acts as a transport medium for cell nutrients and metabolites; for example, the blood transports hormones manufactured in one tissue to target tissue in other parts of the body. Albumin transports bilirubin, the main catabolic residue of hemoglobin, from the spleen to the liver for excretion. Blood urea nitrogen, a nitrogenous waste product, is carried to the kidneys for filtration and excretion. Increased concentration of these normal catabolites can indicate either increased cellular metabolism or a defect in the organ responsible for their excretion. For example, in liver disease, the bilirubin level in blood increases because the liver is unable to function normally and clear the bilirubin. In hemolytic anemia, however, the bilirubin concentration can rise because of the increased metabolism of hemoglobin that exceeds the ability of a normal liver to clear bilirubin.

When body cells die, they release their cellular constituents into surrounding tissue. Eventually, some of these constituents reach the blood. Many constituents of body cells are specific for the cell's particular function; thus, increased concentration of these constituents in the blood, especially enzymes, can indicate abnormal cell destruction in a specific organ.

Blood cells are produced and develop in the bone marrow. This process is known as **hematopoiesis**. Undifferentiated hematopoietic stem cells (precursor cells) proliferate and differentiate under the influence of proteins that affect their function (cytokines). When the cell reaches maturity, it is released into the peripheral blood.

Each of the three cellular constituents of blood has specific functions. Erythrocytes contain the vital protein **hemoglobin**, which is responsible for transport of oxygen and carbon dioxide between the lungs and body tissues. The five major types of leukocytes are neutrophils, eosinophils, basophils, lymphocytes, and monocytes. Each type of leukocyte has a role in defending the body against foreign

pathogens such as bacteria and viruses. Platelets are necessary for maintaining hemostasis. Blood cells circulate through blood vessels, which are distributed throughout every body tissue. Erythrocytes and platelets generally carry out their functions without leaving the vessels, but leukocytes **diapedese** (pass through intact vessel walls) to tissues where they defend against invading foreign pathogens.

CASE STUDY *(continued from page 2)*

1. If Aaron was diagnosed with otitis media, what cellular component(s) in his blood would be playing a central role in fighting this infection?

REFERENCE INTERVALS FOR BLOOD CELL CONCENTRATION

Physiologic differences in the concentration of cellular elements can occur according to race, age, sex, and geographic location; pathologic changes in specific blood cell concentrations can occur as the result of disease or injury. The greatest differences in reference intervals occur between newborns and adults. In general, newborns have a higher erythrocyte concentration than any other age group. The erythrocytes are also larger than those of adults. In the 6 months after birth, erythrocytes gradually decrease in number and then slowly increase. Hemoglobin and erythrocyte counts increase in children between the ages of 5 and 17. The leukocyte concentration is high at birth but decreases after the first year of life. A common finding in young children is an absolute and relative lymphocytosis (increase in lymphocytes). After puberty, males have higher hemoglobin, **hematocrit** (packed red blood cell volume in whole blood), and erythrocyte levels than females. The hemoglobin level decreases slightly after age 70 in males. This is thought to be due to the decrease in testosterone. Tables A through K on the inside covers of this text give hematologic reference intervals for various age groups and by sex if appropriate.

Each individual laboratory must determine reference intervals of hematologic values to account for the physiologic differences of a population in a specific geographical area. Reference intervals for a hematologic parameter are determined by calculating the mean ±2 standard deviations for a group of healthy individuals. This interval represents the reference interval for 95% of normal individuals. A value just below or just above this interval is not necessarily abnormal; normal and abnormal overlap. Statistical probability indicates that about 5% of normal individuals will fall outside the ±2 standard deviation range. The further a value falls from the reference interval, however, the more likely the value is to be abnormal.

CASE STUDY

Aaron's physician ordered a complete blood count (CBC). The results are Hb 11.5 g/dL (115 g/L); Hct 34% (0.34 L/L).

2. What parameters, if any, are outside the reference intervals? Why do you have to take Aaron's age into account when evaluating these results?

HEMOSTASIS

Hemostasis is the property of the circulation that maintains blood as a fluid within the blood vessels and the system's ability to form a barrier (blood clot) to prevent excessive blood loss when the vessel is traumatized, limit the barrier to the site of injury, and dissolve the clot to ensure normal blood flow when the vessel is repaired. Hemostasis occurs in stages called *primary* and *secondary hemostasis* and *fibrinolysis* (breakdown of fibrin). These stages are the result of interaction of platelets, blood vessels, and proteins circulating in the blood. An upset in any of the stages can result in bleeding or abnormal blood clotting (thrombosis). Laboratory testing for abnormalities in hemostasis is usually performed in the hematology section of the laboratory; occasionally, hemostasis testing is performed in a separate specialized section of the laboratory.

☑ CHECKPOINT 1-1

What cellular component of blood can be involved in disorders of hemostasis?

BLOOD COMPONENT THERAPY

Blood components can be used in therapy for various hematologic and nonhematologic disorders. Whole blood collected from donors can be separated into various cellular and fluid components. Only the specific blood component (i.e., platelets for thrombocytopenia or erythrocytes for anemia) needed by the patient will be administered. In addition, the components can be specially prepared for the patient's specific needs (i.e., washed erythrocytes for patients with IgA deficiency to reduce the risk of anaphylactic reactions). Table 1-1 ★ lists the various components that can be prepared for specific uses. The reader may want to refer back to this table when reading subsequent chapters about therapies that use these components.

LABORATORY TESTING IN THE INVESTIGATION OF A HEMATOLOGIC PROBLEM

Laboratory testing is divided into three components: pre-examination, examination, and post-examination (formerly known as preanalytical, analytical, postanalytical). The *pre-examination* component includes all aspects that occur prior to the testing procedure that affect the test outcome such as phlebotomy technique and storage of the specimen after it is drawn but before the test is run. The *examination phase* refers to all aspects affecting the test procedure. The *post-examination* component includes all aspects after the testing is completed such as reporting of results. These three aspects of testing are the backbone of a quality assessment program. See Chapters 10 and 43 for a detailed description of these three phases.

A physician's investigation of a hematologic problem includes taking a medical history and performing a physical examination. Clues provided by this preliminary investigation help guide the physician's choice of laboratory tests to help confirm the diagnosis. The challenge is to select appropriate tests that contribute to a cost-effective and efficient diagnosis. Laboratory testing usually begins

★ **TABLE 1-1** Blood Components and Their Uses

Component Name	Composition	Primary Use
Whole blood	Red blood cells and plasma	Not used routinely; can be used in selected trauma, autologous transfusions, and neonatal situations; increases oxygen-carrying capacity and volume
Packed red blood cells (PRBCs)	PRBCs	Used in individuals with symptomatic anemia to increase oxygen-carrying capability
PRBCs, washed	PRBCs; plasma and most leukocytes and platelets removed	Used for individuals with repeated allergic reactions to components containing plasma and for IgA-deficient individuals with anaphylactic reactions to products containing plasma
PRBCs, leukoreduced	PRBCs; WBC removed	Used to decrease the risk of febrile nonhemolytic transfusion reaction, HLA sensitization, and cytomegalovirus (CMV) transmission
PRBCs, frozen, deglycerolized	PRBCs frozen in cryoprotective agent, thawed, washed	Used for individuals with rare blood groups (autologous donation)
PRBCs, irradiated	PRBCs with lymphocytes inactivated	Used to reduce the risk of graft-vs-host disease
Platelets, pooled[a]	4–6 units of random donor platelets	Used to increase platelet count and decrease bleeding when there is a deficiency or abnormal function of platelets
Platelets, single[a] donor (pheresis)	Equivalent of 4–6 donor platelets collected from single donor	Used to treat patients refractory to random platelet transfusion or to increase platelet count due to a deficiency or abnormal function of platelets
Fresh frozen plasma (FFP)	Plasma with all stable and labile coagulation factors; frozen within 8 hours of collection of unit of blood	Used to treat patients with multiple coagulation factor deficiencies; disseminated intravascular coagulation (DIC); used with packed RBC in multiple transfusions
Cryoprecipitated AHF[b]	Concentrated FVIII, fibrinogen, FXIII, von Willebrand factor	Used to treat patients with hypofibrinogenemia, hemophilia A, von Willebrand's disease, FXIII deficiency
Plasma, cryo-poor	Plasma remaining after cryo removed	Used to treat thrombotic thrombocytopenic purpura (TTP)
Liquid plasma	Plasma not frozen within 8 hours of collection	Used in patients with deficiency of stable coagulation factor(s) and for volume replacement
Granulocytes	Granulocytes	Used to treat the neutropenic patient who is septic and unresponsive to antimicrobials and who has chance of marrow recovery

[a] Platelets can also be leukoreduced or irradiated. See PRBC for reasons.
[b] Cryo = Cryoprecipitated antihemophilic factor

Courtesy of Linda Smith, Ph.D., MLS(ASCP)^{CM}; adapted from the *circular of information for the use of human blood and blood components*. Prepared jointly by the American Association of Blood Banks, America's Blood Centers, and the American Red Cross (2002).

with screening tests; based on results of these tests, more specific tests are ordered. The same tests can be ordered again on follow-up to track disease progression, evaluate treatment, identify side effects and complications, or assist in prognosis.

Hematology screening tests include the **complete blood count (CBC)**, which quantifies the white blood cells (WBCs), red blood cells (RBCs), hemoglobin, hematocrit, and platelets and the **RBC indices** (Chapter 10). The indices are calculated from the results of the hemoglobin, RBC count, and hematocrit to define the size and hemoglobin content of RBCs. The indices are important parameters used to differentiate causes of anemia and help direct further testing. The CBC can also include a WBC differential. This procedure enumerates the five types of WBCs and reports each as a percentage of the total WBC count. A differential is especially helpful if the WBC count is abnormal. When the count is abnormal, the differential identifies which cell type is abnormally increased or decreased and determines whether immature and/or abnormal forms are present, thus providing a clue to diagnosis. The morphology of RBCs and platelets is also studied as a routine part of the differential.

If a hemostasis problem is suspected, the screening tests include the platelet count, **prothrombin time (PT)**, and **activated partial thromboplastin time (APTT)** (Chapter 36). The PT and APTT tests involve adding calcium and thromboplastin or partial thromboplastin to a sample of citrated plasma and determining the time it takes to form a clot. These tests provide clues that guide the choice of follow-up tests to help identify the problem.

Follow-up testing that is done based on results of screening tests is referred to as **reflex testing**. These testing protocols are sometimes referred to as *algorithms*. Follow-up tests can include not only hematologic tests but also chemical, immunologic, and/or molecular analysis. As scientists learn more about the pathophysiology and treatment of hematologic disease and hemostasis, the number of tests designed to assist in diagnosis expands. Errors in selection of the most appropriate laboratory tests and interpretation of results can result in misdiagnosis or treatment errors and is a major source of poor patient outcomes. Laboratory professionals can assist in promoting good patient outcomes by assisting physicians and patient care teams in selecting the most efficient and effective testing strategies[3–5] and assisting in interpretation of test results.[6] Readers are urged to use the reflex testing concept in their thought processes when studying the laboratory investigation of a disease.

In an effort to help the student gain the knowledge to perform these functions, in this text, each hematologic disorder is discussed in the following order: pathophysiology (and etiology, if known), clinical findings, laboratory findings, and treatment. The reader should consider which laboratory tests provide the information necessary to identify the cause of the disorder based on the suspected disorder's pathophysiology. Although it is unusual for the physician to provide a patient history or diagnosis to the laboratory when ordering tests, this information is often crucial to direct investigation and assist in interpretation of the test results. In any case, if laboratory professionals need more patient information to perform testing appropriately, they should obtain the patient's chart or call the physician.

☑ CHECKPOINT 1-2

A 13-year-old female saw her physician for complaints of a sore throat, lethargy, and swollen lymph nodes. A CBC was performed with the following results: Hb 9.0 g/dL (90 g/L); Hct 30% (0.30 L/L); WBC 15×10^9/L; (15×10^3/mL). On the basis of these results, should reflex testing be performed?

Summary

Hematology is the study of the cellular components of blood: erythrocytes, leukocytes, and platelets. Physiological changes in the concentrations of these cells occur from infancy until adulthood. Diseases can upset the steady state concentration of these parameters. A CBC is usually performed as a screening test to determine whether there are quantitative abnormalities in blood cells. The physician can order reflex tests if one or more of the CBC parameters are outside the reference interval. Platelet count, PT, and APTT are screening tests for disorders of hemostasis.

Changes in the health care system focus on containing costs while maintaining quality of care. The laboratory's role in this system is to work with physicians to optimize utilization of laboratory testing.

Review Questions

Level I and Level II

1. In which group of individuals would you expect to find the highest reference intervals for hemoglobin, hematocrit, and erythrocyte count? (Objective 1)
   A. newborns
   B. males older than 12 years of age
   C. females older than 17 years of age
   D. children between 1 and 5 years of age
2. Which cells are important in transporting oxygen and carbon dioxide between the lungs and body tissues? (Objective 2)
   A. platelets
   B. leukocytes
   C. thrombocytes
   D. erythrocytes
3. Forty-five percent of the volume of blood is normally composed of: (Objective 3)
   A. erythrocytes
   B. leukocytes
   C. platelets
   D. plasma
4. Alterations in the concentration of blood cells generally are the result of: (Objective 4)
   A. laboratory error
   B. amount of exercise before blood draw
   C. a disease process
   D. variations in analytical equipment
5. Leukocytes are necessary for: (Objective 2)
   A. hemostasis
   B. defense against foreign pathogens
   C. oxygen transport
   D. excretions of cellular metabolites
6. Laboratories can use which type of testing to help direct the physician's selection of appropriate testing after screening tests are performed? (Objective 5)
   A. reflexive based on results of screening tests
   B. manual repeat of abnormal results
   C. second test by a different instrument
   D. standing orders for all inpatients

7. Screening tests used to evaluate the hemostasis system include: (Objective 6)
   A. PT and APTT
   B. CBC
   C. hemoglobin
   D. WBC count

8. A patient blood specimen is stored in a car for 2 hours with the outside temperature of 95°. This is an example of error in which component of testing? (Objective 8)
   A. pre-examination
   B. examination
   C. post-examination

Companion Resources

http://www.pearsonhighered.com/healthprofessionsresources/

The reader is encouraged to access and use the companion resources created for this chapter. Find additional information to help organize information and figures to help understand concepts.

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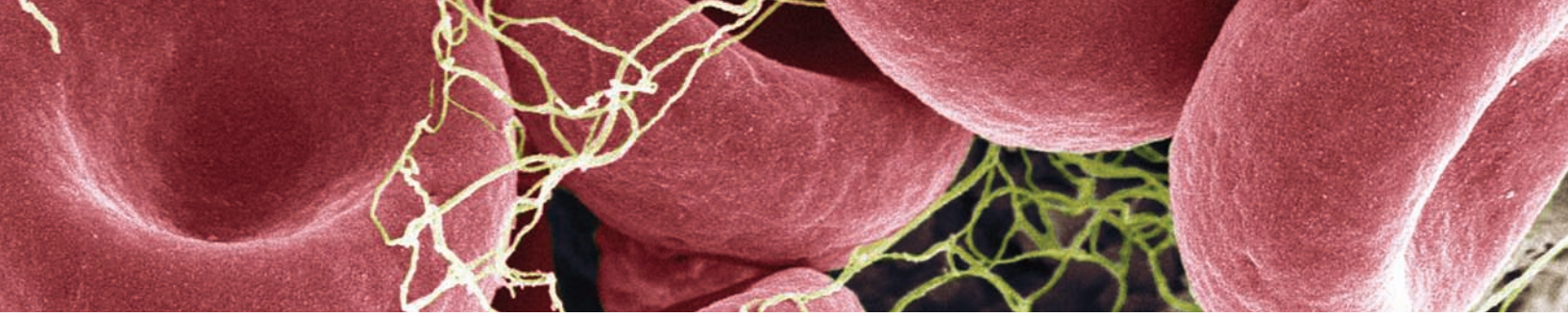


2 Cellular Homeostasis

J. LYNNE WILLIAMS, PHD

Objectives—Level I

At the end of this unit of study, the student should be able to:

1. Describe the location, morphology, and function of subcellular organelles of a cell.
2. Describe the lipid asymmetry found in the plasma membrane of most hematopoietic cells.
3. Differentiate the parts of the mammalian cell cycle.
4. Define R (*restriction point*) and its role in cell-cycle regulation.
5. Define *apoptosis* and explain its role in normal human physiology.
6. Classify and give examples of the major categories of initiators and inhibitors of apoptosis.
7. List the major events regulated by apoptosis in hematopoiesis.

Objectives—Level II

At the end of this unit of study, the student should be able to:

1. Explain the significance of SNPs, introns, exons, UTRs, and post-translational protein modifications.
2. List the components and explain the function of the ubiquitin-proteosome system.
3. Define *cyclins* and *Cdks* and their role in cell-cycle regulation; describe the associated Cdk partners and function of cyclins D, E, A, and B.
4. Define *CAK* (Cdk-activating kinase) and the two major classes of CKIs (cyclin-dependent kinase inhibitors) and describe their function.
5. Compare the function of cell-cycle checkpoints in cell-cycle regulation.
6. Describe/illustrate the roles of p53 and pRb in cell-cycle regulation.
7. Propose how abnormalities of cell-cycle regulatory mechanisms can lead to malignancy.
8. Define *caspases* and explain their role in apoptosis.
9. Differentiate the extrinsic and intrinsic pathways of cellular apoptosis.
10. Define and contrast the roles of pro-apoptotic and anti-apoptotic members of the Bcl-2 family of proteins.

(continued)

Chapter Outline

Objectives—Level II *(continued)*

11. Describe apoptotic regulatory mechanisms.
12. Give examples of diseases associated with increased apoptosis and inhibited (decreased) apoptosis.
13. Define *epigenetics*, and give examples of epigenetic changes associated with gene silencing.
14. Differentiate, using morphologic observations, the processes of necrotic cell death and apoptotic cell death.

Key Terms

Anti-oncogene/tumor suppressor gene
Apoptosis
Caspase
Cell-cycle checkpoint
Cyclins/Cdk
Epigenetics
Exon
Genome/genomics
Intron
Mutation
Necrosis
Polymorphism
Post-translational modification
Proteomics
Proteosome
Proto-oncogene
Quiescence (G_0)
Restriction point (R)
Single nucleotide polymorphism (SNP)
Tissue homeostasis
Transcription factor (TF)
Ubiquitin
Untranslated region (UTR)

Background Basics

LEVEL I AND LEVEL II

Students should have a solid foundation in basic cell biology principles, including the component parts of a cell and the structure and function of cytoplasmic organelles. They should have an understanding of the segments composing a cell cycle (interphase and mitosis) and the processes that take place during each stage.

OVERVIEW

Not all hematology courses include the material in this chapter. It is a review of basic principles of cellular metabolism and homeostasis, which provide the foundation for understanding many pathologic abnormalities underlying the hematologic disorders in subsequent chapters and thus may be of value to some users. The chapter begins with a review of the basic components and cellular processes of a normal cell and presents the concept of tissue homeostasis. Cellular processes that maintain tissue homeostasis—cell proliferation, cell differentiation, and cell death—are discussed at the functional and molecular level. The chapter concludes with a discussion of what happens when genes controlling cell proliferation, cell differentiation, and/or cell death mutate.

INTRODUCTION

The maintenance of an adequate number of cells to carry out the functions of the organism is referred to as **tissue homeostasis**. It depends on the careful regulation of several cellular processes, including cellular proliferation, cellular differentiation, and cell death (apoptosis). A thorough understanding of cell structural components as well as the processes of cell division and cell death allows us to understand not only the normal (physiologic) regulation of the cells of the blood but also disease processes in which these events become dysregulated (e.g., cancer).

CELL MORPHOLOGY REVIEW

A basic understanding of cell morphology is essential to the study of hematology because many hematologic disorders are accompanied by abnormalities or changes in morphology of cellular or subcellular components and by changes in cell concentrations.

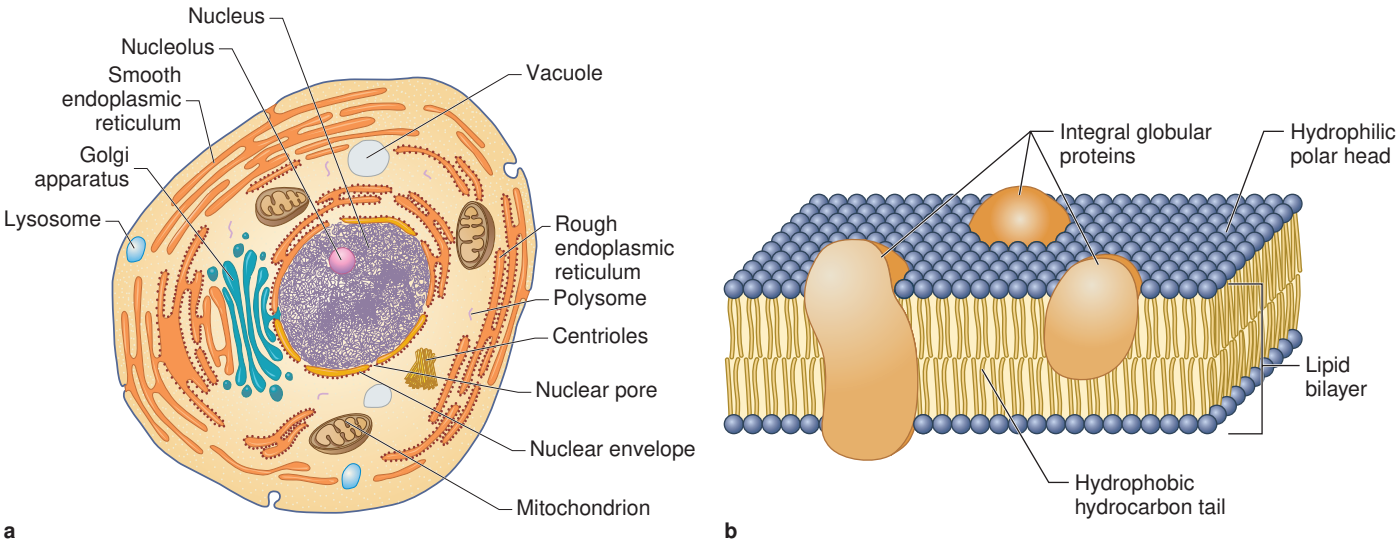
A cell is an intricate, complex structure consisting of a membrane-bound aqueous solution of proteins, carbohydrates, fats, inorganic materials, and nucleic acids. The nucleus, bound by a double layer of membrane, controls and directs the development, function, and division of the cell. The cytoplasm, where most of the cell's metabolic reactions take place, surrounds the nucleus and is bound by the cell membrane. The cytoplasm contains highly ordered organelles, which are membrane-bound components with specific cellular functions (Figure 2-1a ■). The different types of organelles and the quantity of each depend on the function of the cell and its state of maturation.

Cell Membrane

The outer boundary of the cell, the plasma (cell) membrane, is often considered a barrier between the cell and its environment. In fact, it functions to allow the regulated passage of ions, nutrients, and information between the cytoplasm and its extracellular milieu and thus determines the interrelationships of the cell with its surroundings.

The plasma membrane consists of a complex, ordered array of lipids and proteins that serve as the interface between the cell and its environment (Figure 2-1b ■). The plasma membrane is in the form of a phospholipid bilayer punctuated by proteins. The lipids have their polar (hydrophilic) head groups directed toward the outside and inside of the cell and their long-chain (hydrophobic) hydrocarbon tails directed inward. Although the plasma membrane has traditionally been described as a "fluid mosaic" structure,[1] it is in fact highly ordered with asymmetric distribution of both membrane lipids and proteins. The lipid and protein compositions of the outside and inside of the membrane differ from one another in ways that reflect the different functions performed at the membrane's two surfaces.

Four major phospholipids are found in the plasma membrane: phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC), and sphingomyelin (SM) (Web Figure 2-1). Most blood cells have an asymmetric distribution of these phospholipids



■ **FIGURE 2-1** **(a)** Drawing of a cell depicting the various organelles. **(b)** The fluid mosaic membrane model proposed by Singer and Nicholson.

Singer SJ, Nicholson GL. The fluid mosaic model of the structure of cell membranes. *Science*. 1972;175:720–31.

with PE and PS occurring in the inner layer of the lipid bilayer and PC and SM occurring predominantly in the outer layer. The membrane lipids can freely diffuse laterally throughout their own half of the bilayer, or they can flip-flop from one side of the bilayer to the other in response to certain stimuli as occurs in platelets when activated. Membrane lipids including phospholipids, cholesterol, lipoproteins, and lipopolysaccharides contribute to the basic framework of cell membranes and account for the cell's high permeability to lipid-soluble substances. Different mixtures of lipids are found in the membranes of different types of cells.

Although lipids are responsible for the basic structure of the plasma membrane, proteins carry out most of the membrane's specific functions. The proteins of the membrane provide selective permeability and transport of specific substances, structural stability, enzymatic catalysis, and cell-to-cell recognition functions.

The membrane proteins are divided into two general groups: integral (transmembrane) proteins and peripheral proteins. The peripheral proteins are located on either the cytoplasmic or the extracellular half of the lipid bilayer. Some of the integral proteins span the entire lipid bilayer; other integral proteins only partially penetrate the membrane. Some membrane-spanning proteins traverse the membrane once (e.g., erythrocyte glycophorin A) while others cross multiple times (e.g., erythrocyte band 3, the cation transporter). In some cells, such as erythrocytes, peripheral proteins on the cytoplasmic side of the membrane form a lattice network that functions as a cellular cytoskeleton, imparting order on the membrane (Chapter 5).

Carbohydrates linked to membrane lipids (glycolipids) or proteins (glycoproteins) can extend from the outer surface of the membrane. Functions of the carbohydrate moieties include specific binding, cell-to-cell recognition, and cell adhesion. The sugar groups are added to the lipid or protein molecules in the lumen of the Golgi apparatus after synthesis by the endoplasmic reticulum. Many of the glycoprotein transmembrane proteins serve as receptors for extracellular molecules such as growth factors. The binding of the specific ligand to a receptor can result in transduction of a signal to the cell's interior without passage of the extracellular molecule through the membrane (see discussion of cytokine regulation of hematopoiesis, Chapter 4).

Cytoplasm

The cytoplasm, or cytosol, is where the metabolic activities of the cell including protein synthesis, growth, motility, and phagocytosis take place. The structural components, called *organelles*, include the mitochondria, lysosomes, endoplasmic reticulum (ER), Golgi apparatus, ribosomes, granules, microtubules, and microfilaments (Table 2-1 ★). Organelles and other cellular inclusions lie within the cytoplasmic matrix. The composition of the cytoplasm depends on cell lineage and degree of cell maturity. The appearance of cytoplasm in fixed, stained blood cells is important in evaluating the morphology, classifying the cell, and determining the stage of differentiation. Immature or synthetically active blood cells stained with Romanowsky stains (Chapter 37) have very basophilic (blue) cytoplasm due to the large quantity of ribonucleic acid (RNA) they contain.

☑ CHECKPOINT 2-1

What does the phrase *lipid asymmetry* mean when describing cell membranes?

Nucleus

The nucleus contains the genetic material, deoxyribonucleic acid (DNA), responsible for the regulation of all cellular functions. The nuclear material, chromatin, consists of long polymers of nucleotide subunits (DNA) and associated structural proteins (histones) packaged into chromosomes. The total genetic information stored in an organism's chromosomes constitutes its **genome**. The fundamental subunit of chromatin is the nucleosome, a beadlike segment of chromosome composed of about 180 base pairs of DNA wrapped around a histone

★ **TABLE 2-1** Cellular Organelles

Structure	Composition	Function
Ribosomes	RNA + proteins	Assemble amino acids into protein
"Free"	Scattered in the cytoplasm Linked by mRNA-forming polyribosomes	Synthesis of protein destined to remain in cytosol
"Fixed"	Ribosomes bound to outer surface of rough ER	Synthesis of protein destined for export from the cell
ER	Interconnecting membrane-bound tubules and vesicles	Synthesis and transport of lipid and protein
Rough ER	Studded on outer surface with ribosomes	Abundant in cells synthesizing secretory protein; protein transported to Golgi
Smooth ER	Lacks attached ribosomes	Lipid synthesis, detoxification, synthesis of steroid hormones
Golgi apparatus	Stacks of flattened membranes located in juxtanuclear region	Protein from rough ER is sorted, modified (e.g., glycosylated), and packaged; forms lysosomes
Lysosomes	Membrane-bound sac containing catalase, peroxidase, other metabolic enzymes	Destruction of phagocytosed material (extracellular proteins, cells) and cellular organelles (autophagy)
Peroxisome	Membrane-bound sacs containing hydrolytic enzymes	Catabolism of long-chain fatty acids; detoxification of toxic substances
Mitochondria	Double-membrane organelle; inner folds (cristae) house enzymes of aerobic metabolism	Oxidative phosphorylation (ATP production) abundant in metabolically active cells
Cytoskeleton	Microfilaments, intermediate filaments, and microtubules	Gives cell shape, provides strength, and enables movement of cellular structures
Microfilaments	Fine filaments (5–9 nm); polymers of actin	Control shape and surface movement of most cells
Intermediate filaments	Ropelike fibers (~10 nm); composed of a number of fibrous proteins	Provide cells with mechanical strength
Microtubules	Hollow cylinders (~25 mm); composed of protein tubulin	Important in maintaining cell shape and organization of organelles; form spindle apparatus during mitosis
Centrosome	"Cell center"; includes centrioles	Microtubule-organizing center; forms poles of mitotic spindle during anaphase
Centrioles	Two cylindrical structures arranged at right angles to each other; consist of nine groups of three microtubules	Enable movement of chromosomes during cell division; self-replicate prior to cell division

ER = endoplasmic reticulum

protein. The linear array of successive nucleosomes gives chromatin a "beads-on-a-string" appearance in electron micrographs.

The appearance of chromatin varies, presumably depending on activity. The dispersed, lightly stained portions of chromatin (euchromatin) are generally considered to represent unwound or loosely twisted regions of chromatin that are transcriptionally *active*. The condensed, more deeply staining chromatin (heterochromatin) is believed to represent tightly twisted or folded regions of chromatin strands that are transcriptionally *inactive*. In addition to being less tightly associated with the histones, active chromatin characteristically has "unmethylated" promoter regions and highly acetylated histones (see the later section "Epigenetics"). The ratio of euchromatin to heterochromatin depends on cell activity with the younger or more active cells having more euchromatin and a finer chromatin appearance microscopically.

The nuclei of most active cells contain one to four pale staining nucleoli. The nucleolus (singular) consists of RNA and proteins and is believed to be important in RNA synthesis. The nucleolus of very young blood cells is easily seen with brightfield microscopy on stained smears.

A double membrane, the nuclear envelope, surrounds the nuclear contents. The outer membrane (cytoplasmic side) is continuous with the ER and has a polypeptide composition distinct from that of the inner membrane. The gap between the two membranes (~50 nm) is called the *perinuclear space*. The nuclear envelope is interrupted at irregular intervals by openings consisting of nuclear pore complexes (NPCs), which provide a means of communication between nucleus and cytoplasm. NPCs constitute envelope-piercing channels that function as selective gates that allow bidirectional movement of molecules. The nucleus exports newly assembled ribosomal subunits while importing proteins such as transcription factors and DNA repair enzymes.

☑ CHECKPOINT 2-2

Explain the difference between densely staining chromatin and lighter staining chromatin when viewing blood cells under a microscope.

CELLULAR METABOLISM: DNA DUPLICATION, TRANSCRIPTION, TRANSLATION

Genomics is the study of the entire genome of an organism. *Functional genomics* is the study of the actual gene expression "profile" of a particular cell at a particular stage of differentiation or functional activity (i.e., which genes are actively producing mRNA). The morphologic and functional differences between various types of blood cells are governed by which genes are being transcribed/translated into cellular proteins. Microarray or expression array technology can be used to determine the mRNA profile being produced by a cell or tissue of interest, which would reflect which genes are actively being transcribed. The field of

proteomics is the study of the composition, structure, function, and interaction of the proteins being produced by a cell. (Genetic nomenclature has various rules for gene and protein font styles. To differentiate between the gene and its protein, genes are written as italicized capital letters [e.g., *RB*], and the gene's protein product is written with only the first letter capitalized and not italicized. This style is used in this text.)

Genes contain the genetic information of an individual and are found at specific sites on specific chromosomes (gene loci). Most genes are not composed of continuous stretches of nucleotides but are organized into segments called **exons**, which are separated by intervening sequences called **introns**. The exons contain the nucleotide sequences corresponding to the final protein product, while the nucleotide base pairs of the introns do not code for protein. When a gene is transcribed into RNA, the entire sequence of exons and introns is copied as *pre-messenger RNA* (sometimes called *heteronuclear RNA/hnRNA*). Subsequently, the nucleotides corresponding to the intron sequences are spliced out, resulting in the shorter, mature mRNA. Several inherited hematologic diseases, such as some of the thalassemias, result from mutations that derange mRNA splicing (Chapter 14).

Not all of the mature mRNA will be translated into protein. Both the 5′ and the 3′ ends of the mature mRNA encoding the protein to be produced contain **untranslated regions (UTRs)**, which influence the stability of the mRNA and the efficiency of translation to protein. These regions play an important role in regulating many cellular proteins, including those involved in iron metabolism in developing erythrocytes (Chapter 12).

Sometimes large genes with multiple exons can be "read" in a variety of ways, a process described as alternative transcription of the gene. Several different mRNAs and proteins can be produced from a single gene by selective inclusion or exclusion of individual exons from the mature mRNA (i.e., alternative splicing of the pre-mRNA). The human genome is estimated to contain ~30,000 genes; however, alternate transcription and alternate splicing allow for greater genetic complexity than the number of genes would suggest.

Different individuals do not have identical DNA sequences. When a cell replicates its DNA during S phase of the cell cycle (discussed later), the process is not error-free. It has been estimated that ~0.01% of the 6 billion base pairs are copied incorrectly during each S phase.[2] The process of DNA replication is coupled with DNA repair systems to make sure that errors in copying are corrected. If these errors cannot be corrected, the cell may activate its internal apoptotic mechanism (discussed later), resulting in cell death. Errors in DNA replication that cannot be corrected and that subsequently result in activation of apoptosis are believed to be the underlying basis for the large degree of ineffective erythropoiesis in megaloblastic anemias (Chapter 15). In addition to correcting copying errors, DNA repair mechanisms correct other damage to DNA that might have occurred. Failure of these DNA repair mechanisms often contribute to acquired mutations resulting in the development of a malignancy (Chapter 23).

If the miscopied base pair is not corrected, a mutation (or new polymorphism) can occur. Variations in the nucleotide sequence of a gene that can be seen in different individuals are called *alleles*. **Polymorphism** is the term used to describe the presence of multiple alternate copies (alleles) of a gene. Not every alteration in DNA produces an abnormality. For instance, many of the alternate alleles identified for human globin chains do not result in any abnormality of function (Chapter 13). Generally, if the change in DNA sequence does not result in an abnormality of function, the change is called a *polymorphism*. Often the word **mutation** is used only to describe a deleterious change in a gene (e.g., the β^s globin mutation in sickle cell anemia [Chapter 13]).

A region of DNA that differs in only a single DNA nucleotide is called a **single nucleotide polymorphism (SNP)**. SNPs are found at approximately 1 in every 1000 base pairs in the human genome (resulting in ~2.5 million SNPs in the entire genome of a cell). To be considered a true polymorphism, a SNP must occur with a frequency of more than 1% in the general population. If the alteration is known to be the cause of a disease, the nucleotide change is considered to be a mutation rather than a SNP.

Control of Gene Expression

Control of gene expression is a complex process. It must be regulated in both time (e.g., during certain developmental stages) and location (e.g., tissue-specific gene expression). Most genes have a promoter region upstream (5′ side) of the coding region of the gene. **Transcription factors (TFs)** are proteins that bind to the DNA of a target gene's promoter region and regulate expression of that gene. TFs can function to either activate or repress the target gene (some TFs do both, depending on the specific targeted gene). Often TFs are tissue specific, such as GATA-1, a known erythroid-specific TF that regulates expression of glycophorin and globin chains in developing cells of the erythroid lineage.[3]

In addition to the basic on/off function of the promoter region, there are additional layers of control of gene expression. Some genes have enhancer elements or silencer elements, which are nucleotide sequences that can amplify or suppress gene expression, respectively.[2] These response elements influence gene expression by binding specific regulatory proteins (transcription activators, transcription repressors).

Many signals that regulate genes come from outside the cell (e.g., cytokine control of hematopoiesis; Chapter 4). The external molecule or ligand (cytokine) binds to its specific receptor on the surface of the cell. The binding of ligand to receptor activates the receptor and initiates a cell-signaling pathway that conveys the activation signal from the receptor to the nucleus. The end result is an interaction with DNA (e.g., TF binding to one or more gene promoter regions) that either activates or represses the target gene(s).

Protein Synthesis and Processing

Synthesis of proteins (polypeptides) occurs on ribosomes. The newly formed polypeptides are transported to their eventual destination through a sorting mechanism within the cytoplasm.[4] If the polypeptide lacks a "signal sequence," translation is completed in the cytosol, and the protein either stays in the cytosol or is incorporated into the nucleus, mitochondria, or peroxisomes. A polypeptide that contains a signal sequence is extruded into the lumen of the ER (which ultimately gives rise to the more distal structures of the secretory apparatus: the Golgi, endosomes, lysosomes, plasma membrane) (Figure 2-2 ■).

Following import into the ER, proteins undergo appropriate folding and possibly **post-translational modifications**. These are modifications in protein structure that occur after the protein is produced by translation on the ribosome. These changes include the addition of nonprotein groups (such as sugars or lipids), modification of existing amino acids (such as the γ-carboxylation of glutamic acid residues on certain coagulation proteins; Chapter 32) or cleavage of the initial

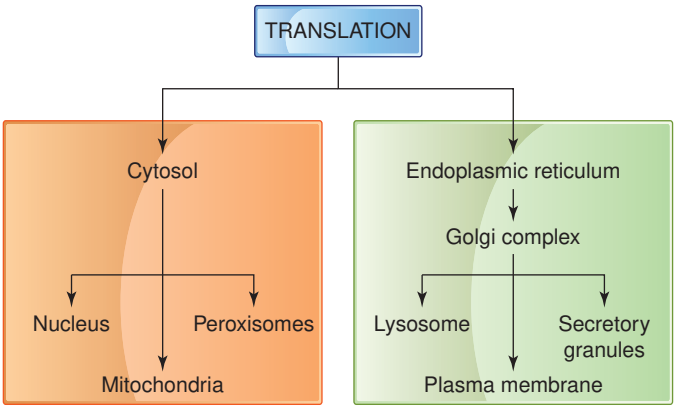


FIGURE 2-2 Proteins are synthesized on ribosomes in the cytoplasm and are targeted for two different pathways. If there is no signal sequence on the polypeptide, translation is completed in the cytosol and the protein is incorporated into the nucleus, mitochondria, peroxisomes, or remains in the cytosol. Polypeptides that have a signal sequence are extruded into the endoplasmic reticulum and are routed to the distal secretory apparatus—the Golgi, lysosomes, endosomes, and plasma membrane.

polypeptide product resulting in a multichain molecule. As the proteins exit the ER, they may be accompanied by molecules that facilitate their transfer from the ER to the Golgi apparatus. A mutation in one of these transfer molecules, ERGIC-53, is the cause of the hemostatic disorder Combined Factor V and VIII deficiency (Chapter 34). During transport through the Golgi, additional processing of the protein can occur.

The primary structure of a protein is defined by its amino acid sequence (see Web Table 2-1 for review of the amino acids and their shorthand notations). A protein emerges from the ribosome in an extended, linear conformation. Subsequently, local regions are folded into specific conformations, the protein's secondary structure, determined by the primary amino acid sequence. The two major secondary protein structures are α-helices and β-pleated sheets. Most proteins are made up of combinations of regions of α-helices and β-pleated sheets connected by regions of less regular structure; these regions are called *loops*. *Molecular chaperones* are cytoplasmic proteins that assist the polypeptide in this folding process. The tertiary structure of a protein refers to its unique three-dimensional shape determined by the folding of secondary structures. Sometimes appropriately folded protein monomers are assembled with other proteins to form multisubunit complexes (also facilitated by chaperones). The quaternary structure of a protein refers to the assembly of independently synthesized polypeptide chains into a multimeric protein (e.g., the $\alpha_2\beta_2$ tetramer, which constitutes hemoglobin A; Chapter 6).

Proteins are often described as being made up of *domains*. Frequently, a domain is encoded in a single exon and represents a region of the polypeptide chain that can fold into a stable tertiary structure. The domains of a protein are often used to designate the location of a particular functional or structural attribute.

☑ CHECKPOINT 2-3

What is the difference between a polymorphism and a mutation?

A mutation that alters a protein's amino acid sequence can result in failure to function. Failure to function can result from either a mutation of a critical functional residue (amino acid) or from the substituted amino acid preventing the protein from folding into its proper three-dimensional structure. Improperly folded proteins are marked for destruction and degraded (via the ubiquitin system).

The Ubiquitin System

Cells contain two major systems for degradation of proteins: the lysosomal system, in which proteolysis occurs within the lysosomes, and the ubiquitin system. The ubiquitin system is a nonlysosomal, proteolytic mechanism in the cytoplasm of most cells that is responsible for disposing of damaged or misfolded proteins.[5] In addition, it regulates numerous cellular processes (e.g., cell-cycle progression, cellular differentiation) by the timed destruction of key regulatory proteins (e.g., cyclins, membrane receptors, transcription factors).

Molecules destined for destruction are tagged with a small (76 amino acid) polypeptide called **ubiquitin** (Figure 2-3 ■). Appropriately labeled molecules are then transferred to an ATP-dependent protease complex (the **proteosome**) for destruction. Generally, proteins bearing a single ubiquitin molecule are marked for endocytosis and degradation in lysosomes. Multi-ubiquitinated proteins are marked for destruction by the proteosome, which is assembled into a cylinder through which proteins are channeled for destruction.

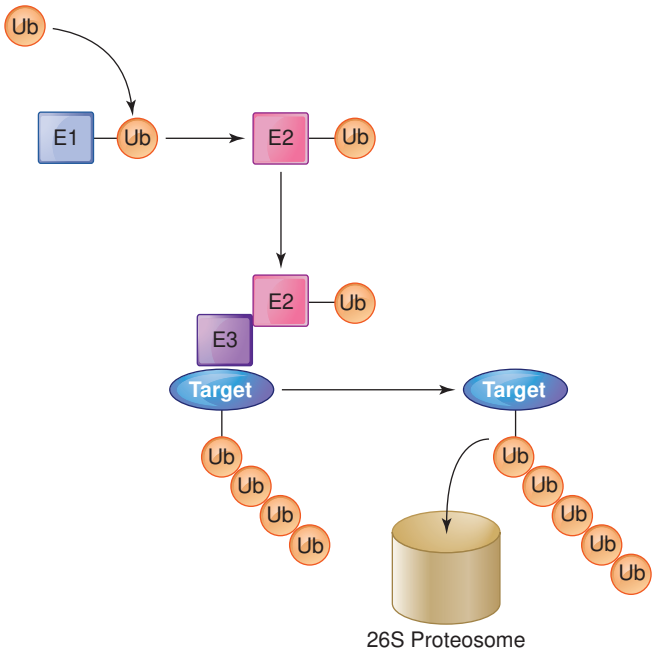


FIGURE 2-3 Ubiquitin-Proteosome system. Ubiquitin-activating enzyme (E1) activates ubiquitin (Ub), which is then transferred to the Ub-conjugating enzyme (E2); Ub ligase (E3) functions in target substrate recognition; it brings together the target and E2-Ub and then catalyzes the transfer of Ub from E2-Ub to the target. Once a target has become multi-ubiquitinated, it is directed to the 26S proteosome for degradation.

TISSUE HOMEOSTASIS: PROLIFERATION, DIFFERENTIATION, AND APOPTOSIS

Tissue homeostasis refers to the maintenance of an adequate number of cells to carry out the organism's functions. In the human body, somatic cells (including blood cells) generally undergo one of three possible fates:

1. Proliferation by mitotic cell division
2. Differentiation and acquisition of specialized functions
3. Death and elimination from the body

Cell proliferation is required for the replacement of cells lost to terminal differentiation, cell death, or cell loss. Differentiation provides a variety of cells, each of which is capable of executing specific and specialized functions. Cell death is also an active process (**apoptosis**) that the cell itself can initiate. Apoptosis is physiologically as important as cell proliferation and differentiation in controlling the overall homeostasis of various tissues. When the regulation of any of these three cellular processes malfunctions or the processes become unbalanced, the consequence may be tissue atrophy, functional insufficiency, or excessive growth/neoplasia (cancer) (Chapters 23–28).

Proliferation: The Cell Cycle

Cell division is required throughout the life of all eukaryotes. Although it has been known for many years that cells have the ability to grow and replicate, the actual mechanisms involved were discovered relatively recently.[6] When a cell is stimulated to divide, it goes through a series of well-defined (biochemical and morphological) stages called the **cell cycle**, which is divided into four phases: G_1 (Gap-1), S (DNA synthesis), G_2 (Gap-2), and M (mitosis) (Figure 2-4 ■).

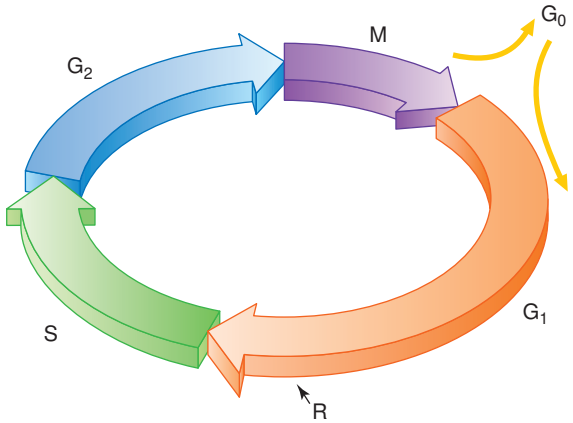
Stages of the Cell Cycle

The physical process of cell division (M phase, or mitosis) includes a series of morphologically recognizable stages (Web Figure 2-2). During mitosis, chromosomes condense (*prophase*) and align on a microtubular spindle (*metaphase*), and sister chromatids segregate to opposite poles of the cell (*anaphase* and *telophase*).

The interval between successive mitoses (known as *interphase*) shows little morphologic variation except that cells increase in volume. During interphase, the cell synthesizes molecules and duplicates its components in preparation for the next mitosis. However, DNA synthesis occurs only within a narrow window of time during interphase. DNA synthesis takes place during *S phase* and is separated from *M phase* (mitosis) by two gap periods: G_1, the time between the end of mitosis and the onset of the next round of DNA replication, and G_2, the time between the completion of S and the onset of mitosis.

Not all of the cells in the body are actively dividing (i.e., actively engaged in the cell cycle). Cells can exit the cell cycle at G_1 and enter a nonproliferative phase called G_0, or **quiescence** (Figure 2-4). To proliferate, a quiescent cell must re-enter the cell cycle. In response to specific mitogenic stimuli or growth factors, quiescent cells can exit G_0 and re-enter the cell cycle at the level of early G_1. In unicellular organisms such as bacteria, cell division depends only on an adequate supply of nutrients. In mammalian cells, all cell division cycles are initiated by specific growth factors or mitogens that drive the cell from G_0 to G_1 ($G_0 \rightarrow G_1$). Some cells, such as terminally differentiated neutrophils, have irreversibly exited the cell cycle during differentiation and are locked in G_0. Other cells, such as hematopoietic stem cells or antigen-specific memory lymphocytes, primarily reside in G_0 but can be induced to return to G_1 and begin cycling with appropriate cytokine or antigen stimulation.

G_1 is characterized by a period of cell growth and synthesis of components necessary for cellular replication. If conditions are unsuitable for proliferation (insufficient nutrients or mitogens) cells will arrest in G_1. As cells transit through the G_1 phase of the cell cycle, they pass through what is called the **restriction point (R)** in late G_1. R defines a point in the cell cycle after which the cell no longer depends on extracellular signals but is committed to completing that cell cycle *independent* of further mitogen stimulation (i.e., cell-cycle completion becomes autonomous).[6] Cells then transit across the G_1/S boundary into S phase where DNA synthesis occurs, followed by the G_2 phase, and finally mitosis where nuclear division (*karyokinesis*) and cytoplasmic separation (*cytokinesis*) occur.



■ **FIGURE 2-4** The four phases of the cell cycle: G_1, S, G_2, and M. G_0 represents the state of quiescence when a cell is withdrawn from the cell cycle. R represents the restriction point—the point in the cell cycle after which the cell no longer depends on extracellular signals but can complete the cycle in the absence of mitogenic stimuli.

Molecular Regulation of the Cell Cycle

The fundamental task of the cell cycle is to faithfully replicate DNA once during the S phase and to distribute identical copies of each chromosome to each daughter cell during M phase. Progression through the cell cycle normally ensures that this takes place, so cells do not initiate mitosis before DNA duplication is completed, do not attempt to segregate sister chromatids until all chromosome pairs are aligned on the mitotic spindle at metaphase, and do not reduplicate their chromosomes (reinitiate S phase) before the paired chromatids have been separated at the previous mitosis. Cells must ensure that chromosome duplication and segregation occur in the correct order (i.e., S → M → S → M). They must also ensure that the next event in the cycle begins *only* when the previous events have been successfully completed (i.e., chromosome duplication is complete before the chromosomes are segregated into the two daughter cells). Entry into and exit from each cell-cycle phase are tightly regulated. Failure to regulate this process results in aneuploidy (abnormal chromosome number)

seen in many malignancies. Research over the past 25 years has begun to reveal how cells guarantee the orderliness of this process.[7]

Cyclins and Cyclin-Dependent Kinases

Enzymatic activities of specific kinases (phosphorylating enzymes) regulate the transition between the various phases of the cell cycle. These kinase proteins (**Cdks**, or cyclin-dependent kinases) phosphorylate target molecules important for cell cycle control. To be active, the kinase (Cdk) must be complexed with a regulatory protein named **cyclin** (hence the name, **c**yclin-**d**ependent **k**inase). The concentration of the different cyclin proteins that regulate the cell cycle rises and falls at specific times during the cell cycle (hence, they are *cycling* proteins). Sequential activation of unique complexes with differing cyclin and Cdk components drive the cell from one cell-cycle stage to the next as summarized in Table 2-2 ★. Each complex in turn phosphorylates key substrates and facilitates or regulates the movement of the cell through the cycle (Figure 2-5a ■). Cdk inhibitors that function to inhibit the kinase activity by binding to the Cdks or the Cdk/cyclin complexes also exist.

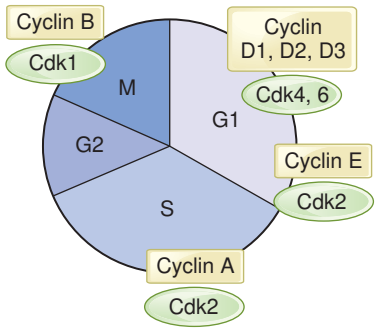
A mammalian cell must receive external signals (growth factors and/or hormones) that trigger the cell to proliferate.[8] These external signals result in an increase of one (or more) of the D cyclins (of which there are three: D1, D2, D3). Cyclin D complexes with Cdk4 or Cdk6 and phosphorylates target molecules required for $G_1 \rightarrow S$ progression. The D cyclins are unique in that they are synthesized in response to growth factor stimulation and remain active in the cell as long as the mitotic stimulus is present. During mid to late G_1, levels of cyclin E increase and bind with Cdk2. The cyclin E/Cdk2 complex is required for the $G_1 \rightarrow S$ transition. Once the cell enters S phase, cyclin E degrades rapidly, and cyclin A takes over the activation of Cdk2. Cyclin A/Cdk2 is required for the onset of DNA synthesis and progression through S phase. Toward the end of S phase, cyclin A starts to activate another kinase, Cdk1, which signals the completion of S phase and the onset of G_2. Cyclin B (which begins to increase during S and G_2) takes over from cyclin A as the activating partner of Cdk1, and cyclin B/Cdk1 controls the onset, sequence of events, and the completion of mitosis. Cyclin B must be destroyed for the cell to exit mitosis and for cytokinesis to take place (Figure 2-5b ■).

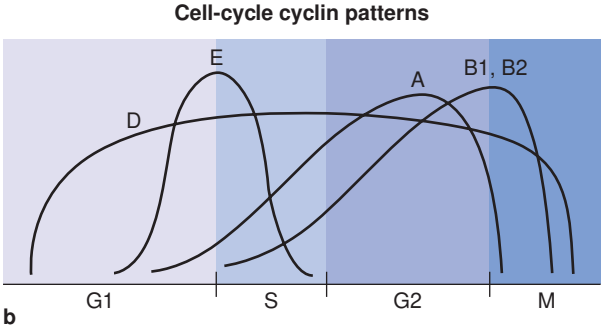
Regulation of Cell-Cycle Kinase Activity

Control of cell-cycle kinase activity is somewhat unique in that protein levels of the enzyme (kinase) subunit remain constant throughout the cell cycle and do not require activation from a proenzyme precursor form. The cell cycle is regulated by altering the availability of the regulatory cofactor (the cyclins) through periodic (and cell-cycle phase-specific) synthesis and degradation of the appropriate cyclin via the ubiquitin system[9] (Figure 2-5b). The periodic accumulation

★ **TABLE 2-2** Cell-Cycle Regulatory Proteins

Cell-Cycle Phase	Cyclin	Partner Cdk
G_1	D1, D2, D3	Cdk4, Cdk6
G_1/S	E	Cdk2
S	A	Cdk2
G_2/M	A	Cdk1
M	B	Cdk1



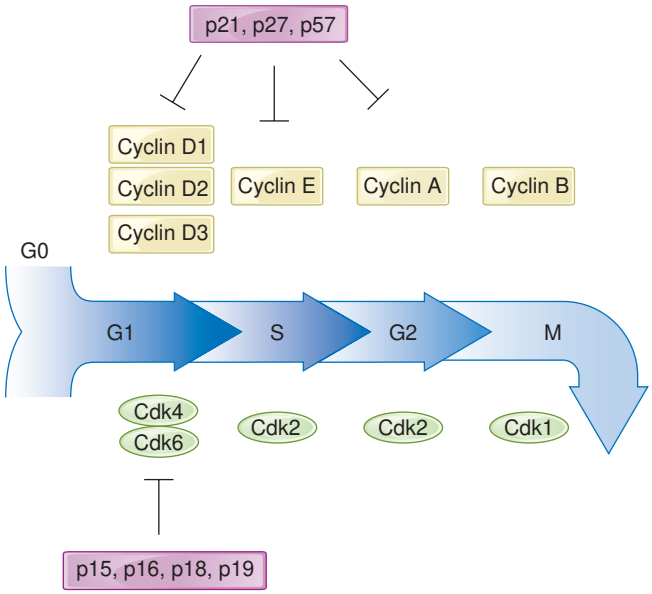


■ **FIGURE 2-5** **(a)** The phases of the cell cycle with the major regulatory cyclin/Cdk complexes depicted for each. **(b)** The levels of the various cyclin proteins during the cell cycle. The cyclins rise and fall in a periodic fashion, thus controlling the cyclin-dependent kinases and their activities.

of different cyclins determines the sequential rise and fall of kinase activities, which in turn regulate the events of cell-cycle progression.

Multiple mechanisms regulate cell-cycle kinase activity. In addition to requiring the appropriate cyclin partner, the kinase subunit (Cdk) must be phosphorylated and/or dephosphorylated at specific amino acid residues in order to have a fully active cyclin/Cdk complex[10] (Web Figure 2-3). The kinase responsible for this activating phosphorylation is CAK (**C**dk-**a**ctivating **k**inase) and is itself a Cdk (Cdk7 complexed with cyclin H). CAK is responsible for activating phosphorylation of *all* the kinases important for mammalian cell-cycle control. On the other hand, phosphorylation of certain amino acids suppresses kinase activity, and these inhibitory phosphates must be removed (by the phosphatase Cdc25) to have a fully active cyclin/Cdk complex (Web Figure 2-3).

The final level of regulation involves two groups of proteins that function as inhibitors of Cdks and cyclin/Cdk complexes[11] (Figure 2-6 ■). The first Cdk inhibitor identified was p21; other Cdk inhibitors with structural and functional similarities to p21 include p27 and p57. (This nomenclature indicates that they are **p**roteins of the indicated molecular mass in kilodaltons [e.g., p21 is a protein of molecular weight of 21,000]). These three inhibitors are considered "universal" because they bind multiple cyclin/Cdk complexes of various phases of the cell cycle (cyclin D/Cdk4/6, cyclin E/Cdk2, and cyclin A/Cdk2). The second group of inhibitors is a family of structurally related proteins that include p15, p16, p18, and p19. These inhibitors are more restricted in their inhibitory activity, inhibit only Cdk4 and Cdk6, and induce cell-cycle arrest in G_1.



■ **FIGURE 2-6** Cdk inhibitors. There are two families of cyclin-dependent kinase inhibitors. The first group, including p15, p16, p18, and p19, inhibits only D-type cyclin/Cdk4 or Cdk6 complexes. The second group of inhibitors, including p21, p27, and p57, possesses a wider spectrum of inhibitory activity, inhibiting the G_1 as well as S phase cyclin/Cdk complexes (cyclin D/Cdk4/6, cyclin E/Cdk2, and cyclin A/Cdk2). ⊥ indicates inhibition of the pathway.

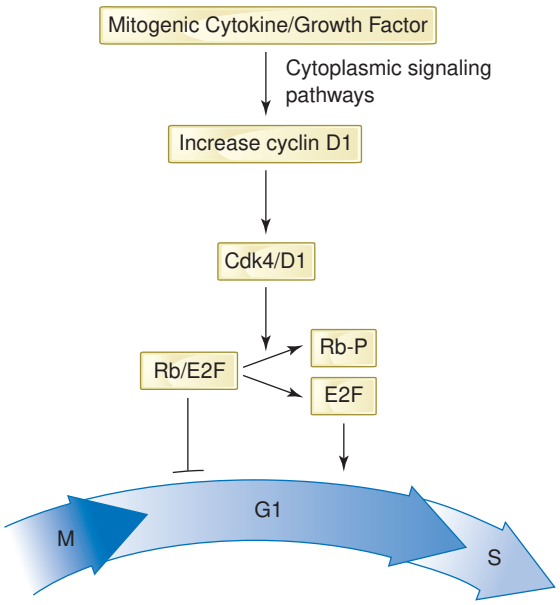
Cell-Cycle Checkpoints

Cell proliferation and differentiation depend on the accurate duplication and transfer of genetic information, which requires the precise ordering of cell-cycle events. Cells achieve this coordination by using **cell-cycle checkpoints** to monitor events at critical points in the cycle and, if necessary, halt the cycle's progression.[12–14] The main functions of checkpoints are to detect malfunctions within the system and to assess whether certain events are properly completed before the cell is allowed to proceed to the next phase of the cycle. When problems are detected, checkpoint mechanisms interrupt cell cycling to allow correction of the problem or elimination of the defective cell.

Four major cell-cycle checkpoints have been described. The *G_1 checkpoint* checks for DNA damage and prevents progression into S phase if the genomic DNA is damaged. The *S-phase checkpoint* monitors the accuracy of DNA replication. The *G_2/M checkpoint* also monitors the accuracy of DNA replication during S phase and checks for damaged or unreplicated DNA; it can block mitosis if any is found. The *metaphase checkpoint* (also called the *mitotic-spindle checkpoint*) functions to ensure that all chromosomes are properly aligned on the spindle apparatus prior to initiating chromosomal separation and segregation at anaphase. If defects are detected at any of these checkpoints, the cell cycle is stopped and repair pathways are initiated, or if the damage is severe and/or irreparable, apoptosis can be triggered (see the section "Apoptosis"). Mechanisms that detect damaged DNA include two important proteins, ATM (**a**taxia-**t**elangiectasia **m**utated) and ATR (**AT** and **R**AD-3-related) kinases.[15] Both are activated by damaged DNA and in turn phosphorylate effectors of the checkpoint response including the proteins Chk1 and Chk2. These proteins in turn activate p53 and/or inhibit Cdc25, which inhibit the cyclin/Cdk complexes or trigger apoptosis.

Two proteins critical for regulation of the cell cycle are p53 and Rb. Rb is the protein product of a gene (*RB*) that predisposes individuals to retinoblastomas and other tumors when only one functional copy of the gene is present. Rb is present throughout the cell cycle, although its phosphorylation state changes markedly at different phases (Figure 2-7 ■).[16, 17] In its *hypo*phosphorylated (active) state, Rb inhibits cell-cycle progression (proliferation) by binding transcription factors (the E2F proteins) that are required for the transcription of genes needed for cell proliferation, thus rendering them nonfunctional. When growth factors induce activation of cyclin D/Cdk4/6, the Rb protein is phosphorylated by this kinase activity. As cells progress through G_1, *hyper*phosphorylation of Rb by cyclin D/Cdk4/6 kinase results in the inactivation of Rb, the release of the active E2F transcription factors, and the activation and expression of genes required for cell-cycle progression. Cyclin E/Cdk2 and cyclin A/Cdk2 subsequently maintain Rb hyperphosphorylation through the cell cycle. *RB* functions as a tumor suppressor gene. Cells that lack functional Rb protein have deregulation of cell-cycle genes and cell proliferation, sometimes resulting in malignancy.

The protein p53 is not required for normal cell function (i.e., it is not *required* for cell-cycle progression). However, it serves an important function as a molecular policeman that monitors the genome's integrity.[18]



■ **FIGURE 2-7** The role of the retinoblastoma susceptibility gene product (Rb) in regulation of the cell cycle. Stimulation of a cell with mitogens or growth factors induces synthesis of the D-type cyclins. Activation of G_1 phase kinase activity (cyclin D/Cdk4/6) phosphorylates a number of intracellular substrates including the Rb protein. In the hypophosphorylated (active) state, Rb binds and sequesters transcription factors known as E2F, rendering them inactive. When cyclin D/Cdk4 or Cdk6 phosphorylates Rb, it releases the E2F transcription factors, which then move to the nucleus, and initiate transcription of genes required for cell-cycle progression (including the genes for cyclin E and cyclin A). ↓ indicates stimulation of the pathway; ⊥ indicates inhibition of the pathway.

p53 is a transcription factor that can both activate and inhibit gene expression, depending on the target gene. It is induced when DNA damage is detected and puts the brakes on cell growth and division. This allows time for DNA repair or can trigger apoptosis if repair is not possible.

Elevated levels of p53 result in upregulation of the Cdk inhibitor p21 and inhibition of the Cdc25 phosphatase (blocking kinase function), induction of pro-apoptotic Bax, and inhibition of anti-apoptotic Bcl-2 (see the section "Apoptosis"). p53 is an important component of both the G_1 and the G_2/M checkpoints. Like *RB, p53* functions as a tumor suppressor gene and is the most commonly mutated gene in human malignancies.

☑ CHECKPOINT 2-4

A cell undergoing mitosis fails to attach one of its duplicated chromosomes to the microtubules of the spindle apparatus during metaphase. The cell's metaphase checkpoint malfunctions and does not detect the error. What is the effect (if any) on the daughter cells produced?

Differentiation

Differentiation is the process that generates the diverse cell populations found throughout the body. All cells in the human body contain the exact same genetic information. The appearance of specific characteristics in various cell populations is dictated by the specific genes that are actively being transcribed into mRNA and the translation of that genetic information into functional proteins. Regulation of gene expression is controlled at various levels.

The transcription of genes is regulated by binding transcription factors to the promoter regions of the genes that encode for proteins specific for the given type of cell, resulting in tissue-specific mRNAs. As differentiation progresses within a given tissue or cell lineage, different genes will be sequentially activated and inactivated, resulting in a changing landscape of mRNAs and proteins that drive the differentiation process. Two additional cell systems, epigenetics and mRNA interference, are important in the regulation of this process.

Epigenetics

Epigenetics (meaning literally "on top of genetics") refers to stable changes in gene function that are passed from one cell to its progeny. Epigenetic changes play an important role in normal development and differentiation and are associated with "silencing" genes and chromatin condensation into heterochromatin.[19]

One of the most common epigenetic changes found in the human genome involves the methylation of certain cytosine nucleotides (C^M) within genes and/or their promoter regions.[20] Cytosine nucleotides found adjacent to a guanine nucleotide, the so-called CpG dinucleotide, are particularly susceptible to methylation.

$$CGATCGATCGAT \rightarrow C^MGATC^MGATC^MGAT$$

These methylations or epigenetic changes become incorporated into the genetic/epigenetic regulatory mechanisms of the cell, are conserved during subsequent cell divisions, and play a significant role in cellular differentiation pathways. The methylation of CpG dinucleotides is a potentially reversible process, and approximately 70–75% of CpG dinucleotides in the human genome are methylated. In addition, CpG dinucleotides are often clustered in *CpG islands*, many of which are in and around the promoter regions of genes. The unmethylated state of a gene's promoter region indicates a *transcription-ready status* and is seen in genes actively being transcribed into mRNA. Typically, methylation of the promoter regions is associated with gene silencing and is part of the normal terminal differentiation process seen in many diverse tissue types.

Extensive information also can be encoded in the protein component of the chromatin in what is called the *histone code*. Modifications of the histone proteins include lysine acetylation, serine phosphorylation, and lysine and arginine methylation.[21] These modifications can also be passed from one cell generation to the next during cell division and play an important role in the complex system responsible for regulating euchromatin to heterochromatin transitions. Hypoacetylated histones bind tightly to the phosphate backbone of DNA and help maintain chromatin in an inactive, silent state. Acetylated histones maintain a more relaxed chromatin structure and allow gene transcription to occur.[22] Both DNA methylation and histone hypoacetylation promote chromatin condensation and gene silencing.

As cells go through a particular differentiation program, the DNA methylation patterns and histone acetylation/deacetylation patterns change as successive genes are activated and deactivated. It is possible to map the DNA methylation patterns within a cell by using a method called *microarray analysis*, and this can be useful in evaluating a variety of diseases, including cancer.

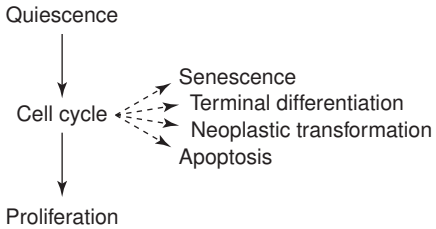
Translational Regulation

Genomic expression in the context of which proteins are produced within a cell is controlled not only at the level of gene transcription (production of mRNA) but also by post-transcriptional events that affect mRNA stability. Interfering with the function of mRNA (RNA interference, RNAi) can also modify the function of genes.[2]

Two forms of RNA are involved in regulating translation of mRNA, micro-RNA (miRNA) and small interfering RNA (siRNA). Both types of molecules function in RNAi pathways and block protein expression by inhibiting translation or inducing degradation of their respective target mRNA (and thus "gene silencing"). The exact mechanisms of RNAi function in normal cellular biology, and the contribution of RNAi to various pathologic states are areas of active ongoing research.

Apoptosis

Cells stimulated to enter the cell cycle can experience outcomes other than proliferation (Figure 2-8 ■). Cells can undergo senescence in which they are permanently growth arrested and no longer respond to mitogenic stimuli. Cells entering the cell cycle can also become



■ **FIGURE 2-8** Alternative fates for a cell induced to enter the cell cycle.

★ **TABLE 2-3** Cardinal Features of Apoptosis and Necrosis

Feature	Necrosis	Apoptosis
Stimuli	Toxins, severe hypoxia, massive insult, conditions of ATP depletion	Physiologic and pathologic conditions without ATP depletion
Energy requirement	None	ATP dependent
Histology	Cellular swelling; disruption of organelles; death of patches of tissue	Cellular shrinkage; chromatin condensation; fragmentation into apoptotic bodies; death of single isolated cells
DNA breakdown pattern	Randomly sized fragments	Ladder of fragments in internucleosomal multiples of 185 base pairs
Plasma membrane	Lysed	Intact, blebbed with molecular alterations (loss of phospholipid asymmetry)
Phagocytosis of dead cells	Immigrant phagocytes	Neighboring cells
Tissue reaction	Inflammation	No inflammation

terminally differentiated (committed) into specialized cell types. Uncontrolled cell cycling is a characteristic feature of malignant cells. Finally, cells can exit at any phase of the cell cycle by undergoing programmed cell death (apoptosis).

Cells can die by either **necrosis** or **apoptosis**. The criteria for determining whether a cell is undergoing apoptosis or necrosis originally relied on distinct morphologic changes in the appearance of the cell[23] (Table 2-3 ★). Necrotic death is induced by lethal chemical, biological, or physical events (extracellular assault). Such a death is analogous to "cell murder." In contrast, apoptosis, or "programmed cell death," is a self-induced death program regulated by the cell itself ("cell suicide").

Apoptosis plays an essential role in the development and homeostasis of all multicellular organisms.[24] Apoptosis helps maintain a constant organ size in tissues that undergo continuous renewal, balancing cell proliferation and cell death. It also occurs at defined times and locations during development.

In adults, apoptosis is also important in tissue homeostasis; homeostasis generally balances generation of new cells with the loss of terminally differentiated cells. Apoptosis is responsible for the elimination of excess cells (such as expanded clones of T or B lymphocytes following immune stimulation, or excess neutrophils following a bacterial challenge). As a defense mechanism, apoptosis is used to remove unwanted and potentially dangerous cells such as self-reactive lymphocytes, cells infected by viruses, and tumor cells. Diverse forms of cellular damage can trigger apoptotic death including DNA damage or errors of DNA replication, which prevent the cell with abnormal DNA from proliferating. Similarly, intracellular protein aggregates or misfolded proteins can stimulate apoptosis (e.g., the ineffective erythropoiesis and intramedullary apoptotic death of erythroblasts in β-thalassemia major triggered by aggregates of α globin chains [Chapter 14]). In addition to the beneficial effects of programmed cell death, the inappropriate activation of apoptosis can cause or contribute to a variety of diseases[25,26] (Table 2-4 ★).

Apoptosis is initiated by three major types of stimuli (Table 2-5 ★):

1. Deprivation of survival factors (growth factor withdrawal or loss of attachment to extracellular matrix)
2. Signals by "death" cytokines through apoptotic "death" receptors (tumor necrosis factor [TNF], Fas ligand)
3. Cell-damaging stress

Conversely, apoptosis is inhibited by growth-promoting cytokines and interaction with appropriate extracellular environmental stimuli. The disruption of cell physiology as a result of viral infections can cause an infected cell to undergo apoptosis. This suicide of

★ **TABLE 2-4** Diseases Associated with Increased and Decreased Apoptosis

Increased Apoptosis	Decreased Apoptosis
AIDS	Cancer
Neurodegenerative disorders	• Follicular lymphomas
• Alzheimer's disease	• Other leukemias/lymphomas
• Parkinson's disease	• Carcinomas with *p53* mutations
• Amyotrophic lateral sclerosis	• Hormone-dependent tumors (breast, prostate, ovarian)
• Retinitis pigmentosa	Autoimmune disorders
Myelodysplastic syndromes	• Systemic lupus erythematosus
Aplastic anemia	• Other autoimmune diseases
Ischemic injury	Viral infections
• Myocardial infarction	• Herpes viruses
• Stroke	• Poxviruses
• Reperfusion injury	• Adenoviruses
Toxin-induced liver disease	

★ **TABLE 2-5** Inhibitors and Initiators/Inducers of Apoptosis

Inhibitors	Initiators/Inducers
Presence of survival factors • Growth factors • Extracellular matrix • Interleukins • Estrogens, androgens	Deprivation of survival factors • Growth factor withdrawal • Loss of matrix attachment
Viral products that block apoptosis • Cowpox virus CrmA • Epstein Barr virus BHRF-1	Death cytokines • TNF • Fas ligand
Pharmacologic inhibitors	Cell-damaging stress • Bacterial toxins • Viral infections • Oxidants • Glucocorticoids • Cytotoxic drugs • Radiation therapy
Oncogene and tumor suppressor gene products (Bcl-2, Bcl-X_L, Mcl-1, Rb, c-Abl)	Oncogene and tumor suppressor gene products (c-myc, p53, Bax, Bad, BCL-X_S, c-Fos, c-Jun)

an infected cell can be viewed as a cellular defense mechanism to prevent viral propagation. To circumvent these host defenses, a number of viruses have developed mechanisms to disrupt the normal regulation of apoptosis within the infected cell. Finally, a number of oncogenes and tumor suppressor genes that can either stimulate or inhibit apoptosis have been described (Chapter 23).

Necrosis versus Apoptosis

When a cell is damaged, the plasma membrane often loses its ability to regulate cation fluxes, resulting in the accumulation of Na^+, Ca^{++}, and water (Table 2-3). Consequently, the necrotic cell exhibits a swollen morphology. The organelles also accumulate cations and water, swell, and ultimately lyse. The rupture of the cytoplasmic membrane and organelles releases cytoplasmic components (including proteases and lysozymes) into the surrounding tissue, triggering an inflammatory response. In contrast, apoptosis is characterized by cellular shrinking rather than swelling, with condensation of both the cytoplasm and the nucleus. Apoptotic cells do not lyse, but portions of the cells pinch off as apoptotic bodies that are phagocytized by neighboring cells or macrophages. Thus, apoptosis is a very efficient process by which the body can remove a population of cells at a given time or in response to a given stimulus without the activation of an inflammatory response.

Necrosis is a passive event induced by the external injurious agent and generally leads to the destruction of a large group of cells in the same area. In contrast, apoptosis is an energy-dependent process orchestrated by the cell itself and generally affects only individual cells. In addition, a particular type of DNA fragmentation characterizes apoptosis. DNA in an apoptotic cell is enzymatically cleaved by a specific endonuclease into oligonucleotides whose sizes are multiples of ~ 185 base pairs (corresponding to nucleosomal fragments). When electrophoresed on agarose gel, these nucleotide fragments make a discrete "ladder pattern" that is considered the hallmark of apoptosis. This is in contrast to the "smudge" pattern seen in cells undergoing necrosis, which indicates the presence of randomly degraded DNA.

Molecular Regulation of Apoptosis

Apoptosis is a closely regulated physiologic process that involves a group of proteins called *caspases* and the *Bcl-2 family of proteins*.

Role of Caspases and the Initiation of Apoptosis

The cellular events responsible for apoptotic cell death are directed by **caspases**,[27,28] a family of **c**ysteine proteases that cleave after **asp**artic acid amino acids in a peptide substrate and are responsible for the orderly dismantling of the cell undergoing apoptosis.

At least 14 caspase enzymes (caspase 1–14) have been identified in humans, although not all play a significant role in apoptosis. Those that are involved in apoptosis form the effector arm of the apoptotic machinery that, once activated, carries out the proteolysis necessary for apoptosis to occur. Caspases are found in healthy cells as zymogens (inactive form) and express their protease activity following either proteolytic cleavage or autocatalytic activation at high concentrations. A hierarchical relationship similar to that described for the blood coagulation proteins exists among the apoptotic caspases. Early acting, initiator caspases (e.g., caspase-2, -8, -9, -10) are recruited in response to apoptotic stimuli and are activated. They then initiate the apoptotic cascade by proteolytically activating downstream effector caspases (e.g., caspase-3, -6, -7), which in turn orchestrate the cell's death[28,29] (Figure 2-9 ■). Activation of caspases in apoptosis does not lead to indiscriminate proteolytic degradation but to specific cleavage of key substrates including proteins involved in cell structure, cell-cycle regulation, transcription, translation, DNA repair, and RNA splicing. One key substrate activated by caspases is an endonuclease (CAD/**c**aspase-**a**ctivated **D**NAse) that is responsible for the characteristic DNA fragmentation (Web Table 2-2).

Two major cell death pathways (Web Figure 2-4) are initiated by a variety of events. Similar to the coagulation cascade (Chapter 32), there is an "extrinsic pathway" and an "intrinsic pathway." The extrinsic pathway is triggered by extracellular signals ("death cytokines") and transmitted through "death receptors" on the surface of the cell. The intrinsic pathway is a mitochondria-dependent pathway initiated by intracellular signals in response to stress, exposure to cytotoxic agents, DNA damage, or radiation.

At least eight death receptors have been described in mammalian cells to date.[30–32] The two best-known death cytokines and death receptors (DR) are (1) tumor necrosis factor (TNF) and the TNF receptor and (2) Fas Ligand and CD95 (Fas receptor). DRs do not bind initiator caspases directly but interact through adapter molecules containing "docking sites" or domains for each protein (Web Figure 2-5). Once the

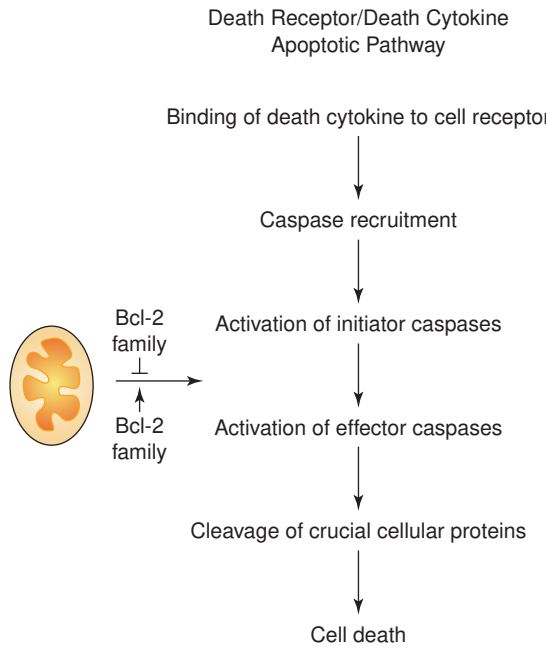


FIGURE 2-9 The apoptotic pathway triggered by death cytokine binding to death receptors. Activation of a death receptor by binding of death cytokine results in the recruitment of specific adapter proteins and activation of initiator caspases. Activated initiator caspases can then proceed to activate downstream effector caspases that mediate the cleavage of various cellular proteins during apoptosis. The contribution of the Bcl-2 family of pro-apoptotic and anti-apoptotic proteins in determining whether activation of initiator caspases will proceed through to activation of effector caspases is depicted. ↓ indicates stimulation of the pathway; ⊥ indicates inhibition of the pathway.

death cytokine, death receptor, adapter molecules, and initiator caspases are assembled in a complex called DISC (**D**eath-**I**nducing **S**ignaling **C**omplex), the caspase is activated by the process of autocatalysis.

The sequence of events triggering apoptosis via the intrinsic pathway is less understood. It involves the assembly of a second caspase-activating complex called the *apoptosome* (Web Figure 2-5). DNA damage, triggering cell-cycle checkpoints, or loss of survival factors increase expression of pro-apoptotic Bcl proteins (discussed in the section "Role of Bcl-2 Proteins") and trigger mitochondrial release of cytochrome-c that serves as a cofactor for caspase activation. Cytochrome-c assembles with a different adapter protein and initiator caspase, again triggering autocatalysis. The activated initiator caspases from both pathways converge on the proteolytic activation of the effector caspase, caspase-3, and trigger apoptosis.

Role of Bcl-2 Proteins

The Bcl-2 family of proteins includes both pro-apoptotic and anti-apoptotic members and constitutes a critical intracellular checkpoint regulating apoptosis.[33,34] The founding member, Bcl-2, was a protein originally cloned from B-cell lymphomas with the characteristic t(14;18) chromosomal translocation (Chapter 28). Since that initial discovery, several additional proteins related to Bcl-2 have been identified, some of which promote and others oppose apoptosis. At present, there are at least 6 known apoptosis-inhibitory proteins (survival factors) including the originally described Bcl-2 and at least 14 pro-apoptotic family members.[32] The Bcl-2 family of proteins is localized at or near the mitochondrial membranes and constitutes a critical intracellular checkpoint of apoptosis. Bcl protein interactions determine whether early activation of initiator caspases proceeds to full activation of effector caspases (see Figure 2-9).[35,36]

The relative levels of anti-apoptotic and pro-apoptotic Bcl-2 family members constitute a *rheostat* for apoptosis. This rheostat is regulated by different associations between prosurvival and prodeath proteins, all of which share similar structural regions that allow them to form dimers or higher oligomers (either homo- or hetero-oligomers). Bax, the first pro-apoptotic member discovered, can associate with itself (Figure 2-10 ■); Bax:Bax homo-oligomers promote apoptosis. They induce permeabilization of the mitochondrial membrane and release of proteins, including cytochrome-c, and activation of the caspase cascade. When Bcl-2 is increased, Bax:Bcl-2 hetero-oligomers form and repress apoptosis. Actually, it is the overall ratio of various death agonists (Bax and related proteins) to death antagonists (Bcl-2 and related proteins) and their interactions with each other that determine the susceptibility of a cell to a death stimulus (Figure 2-11 ■).

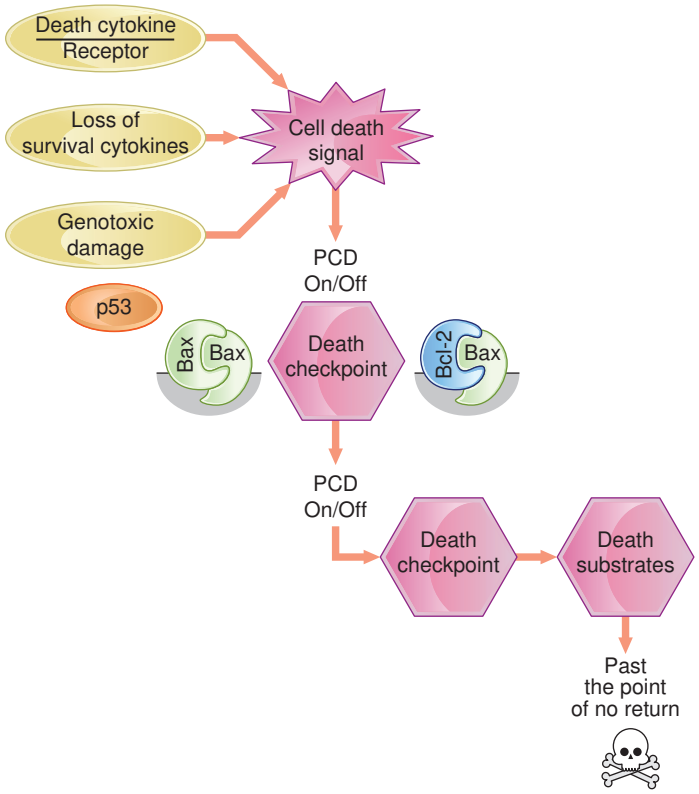
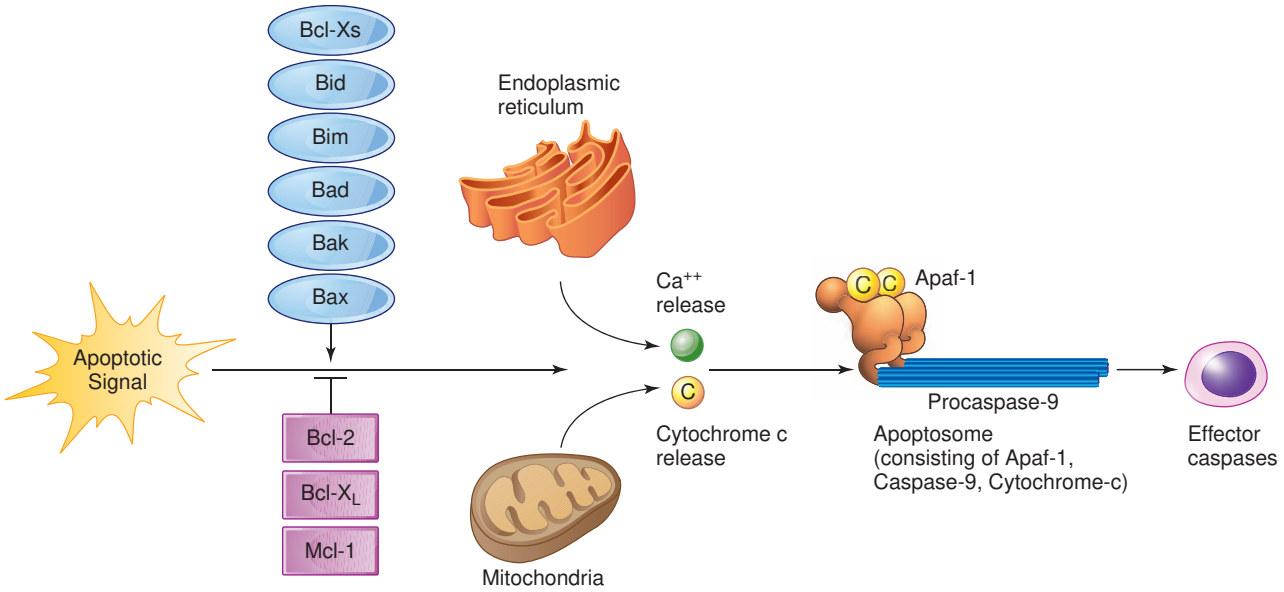


FIGURE 2-10 Model of cell death checkpoints. Following delivery of a cell death signal (genotoxic damage, loss of survival cytokines, or presence of death cytokines), the ratio of pro-apoptotic components (Bax and related molecules) versus anti-apoptotic components (Bcl-2 and related molecules) determines whether the death program will continue to completion. A preponderance of Bax:Bax homodimers promotes continuation of the process while Bax–Bcl-2 heterodimers shuts it down.

PCD = programmed cell death (apoptosis)



■ **FIGURE 2-11** Bcl-2–related proteins and control of apoptosis. Pro-apoptotic (blue ovals) and anti-apoptotic (pink rectangles) Bcl-2–related proteins interact in response to an apoptotic signal. If the pro-apoptotic signals prevail, cytochrome-c (yellow circle) is released from the mitochondria, binds to an adapter protein (Apaf-1), and recruits an initiator caspase (procaspase-9); the resulting caspase-activating assembly, the apoptosome, is associated with the intrinsic pathway of apoptosis.

The cell receives and processes *death signals* from a variety of sources. They converge on this rheostat, which determines whether the cell will activate effector caspases and subsequently whether there will be cleavage of the *death substrates* necessary for apoptosis.[34]

Cells utilize a variety of safeguards to prevent inappropriate apoptosis. They possess a number of proteins that modulate cell death by binding to activated caspases and interfering with their activity, the so-called **i**nhibitors of **a**poptosis **p**roteins (IAPs).[27,28] Some viruses contain viral proteins that perform the same function (e.g., cowpox viral protein CrmA, Adenovirus E1B, Baculovirus p35). These viral proteins block the apoptosis-activating defense of the host cell against viral replication (i.e., block apoptosis).

Apoptosis and the Hematopoietic System

Apoptosis is important in the hematopoietic system (Table 2-6 ★). The default cellular status of hematopoietic precursor cells is *cell death* (Chapter 4). Cytokines and components of the extracellular matrix function to suppress apoptosis, allowing survival of hematopoietic cells when appropriate cytokines are present. Apoptosis plays an essential role in the selection of appropriate recognition repertoires of T and B lymphocytes, eliminating those with nonfunctional or autoreactive antigen receptors (Chapter 8). Apoptosis helps regulate the overall number of mature cells by inducing elimination (cell death) of excess cells when expanded numbers of mature cells are no longer needed (i.e., expanded T- and B-cell clones following elimination of foreign antigen and elimination of neutrophils, eosinophils, and monocytes following an infection/inflammatory response). Apoptosis is the mechanism employed in cytotoxic killing by cytotoxic T lymphocytes (CTL) and natural killer (NK) cells. Finally, activation of apoptotic caspases is involved in platelet production and release from mature megakaryocytes and in the final stages of erythrocyte maturation (chromatin condensation and organelle removal).[37,38]

Dysregulation of apoptosis also contributes to hematologic disorders. Apoptosis is increased in the myelodysplastic syndromes and tends to be decreased in the acute leukemias, perhaps partly explaining the pancytopenias and leukocytosis, respectively, seen in those disorders (Chapters 25–27).

☑ CHECKPOINT 2-5

What would be the effect on the hematopoietic system homeostasis if the expanded clone of antigen-activated B lymphocytes failed to undergo apoptosis after the antigenic challenge was removed?

★ **TABLE 2-6** The Role of Apoptosis in the Hematopoietic and Lymphoid Systems

I. Default cellular status for hematopoietic stem cells and progenitor cells
Apoptosis regulated by cytokines and extracellular matrix
II. Lymphoid homeostasis
Selection of recognition repertoires of T and B cells
Elimination of autoreactive lymphocytes
Downregulation of immune response following antigen stimulation
Cytotoxic killing by CTL and NK cells
III. Elimination of eosinophils, monocytes, and neutrophils following infection/inflammatory response
IV. Developmental pathways for erythropoiesis and thrombopoiesis

CTL = cytotoxic T lymphocytes; NK = natural killer