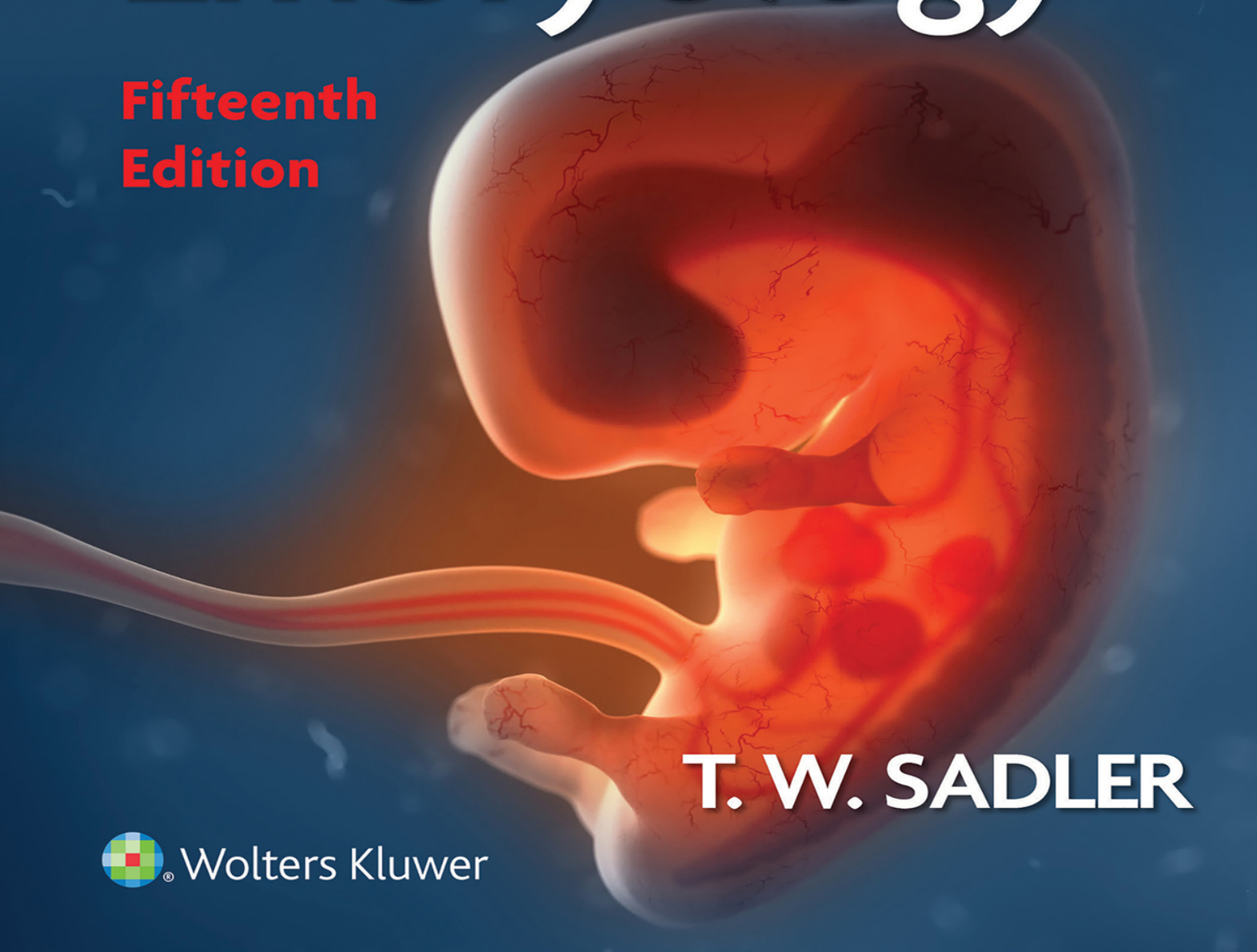
LANGMAN'S Medical Embryology

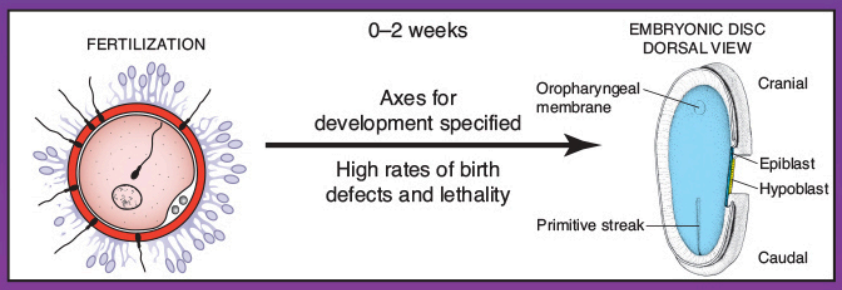
Fifteenth Edition

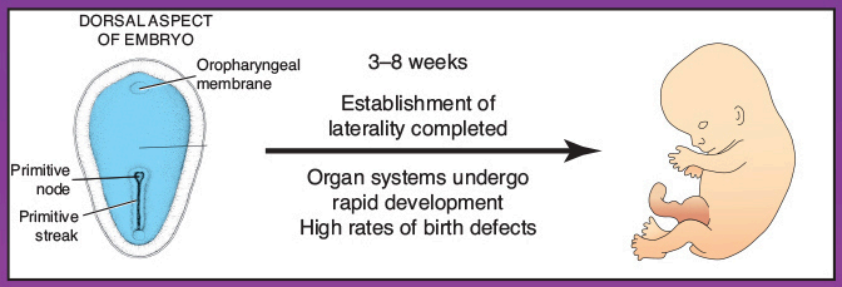


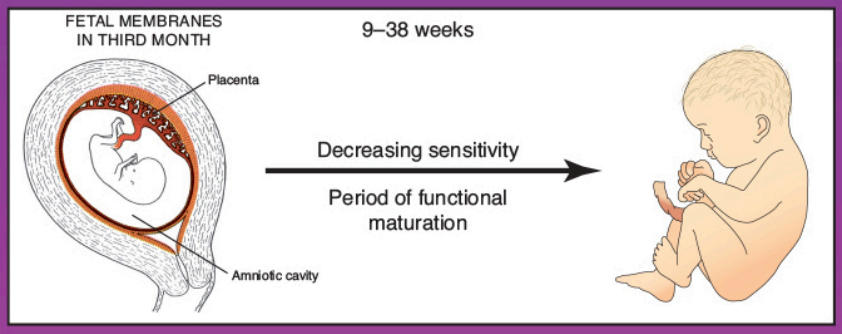
T. W. SADLER

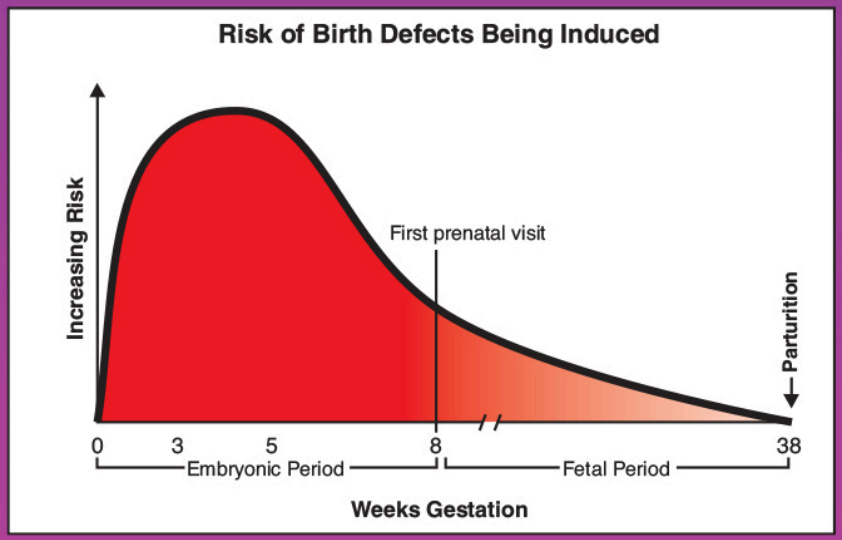
Wolters Kluwer

Periods of Susceptibility to Teratogenesis



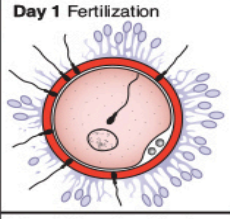




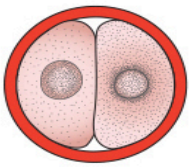


Embryonic Development in Days

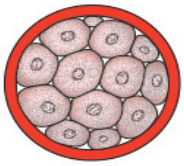
Day 1 Fertilization



Day 2 Two-cell stage

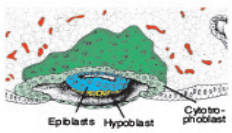


Day 3 Morula

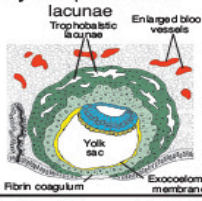


Day 4 Early blastocyst

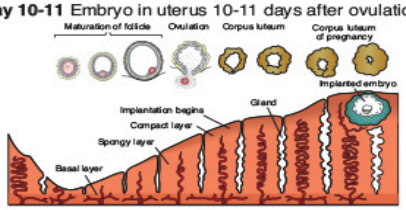
Day 8 Bilaminar disc forms



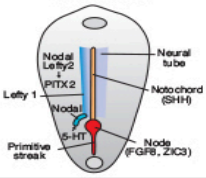
Day 9 Trophoblast with lacunae



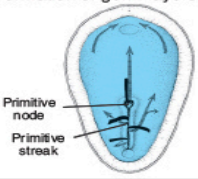
Day 10-11 Embryo in uterus 10-11 days after ovulation



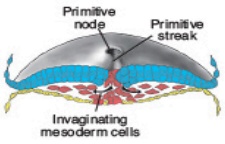
Day 15 Laterality established



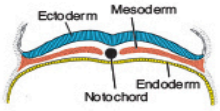
Day 16 Gastrulation: Formation of germ layers



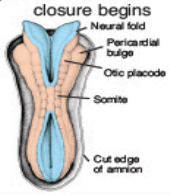
Day 17 Epiblast forms germ layers



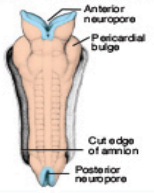
Day 18 Trilaminar embryonic disc



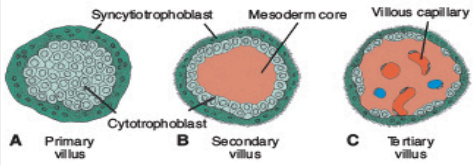
Day 22 Neural tube closure begins



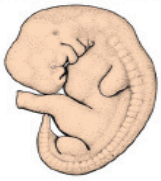
Day 23 Neural tube zippers



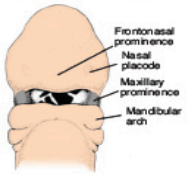
Day 24-25 Villus formation continues in the placenta



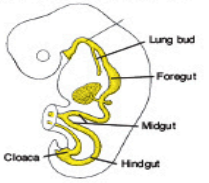
Day 29 Arm and leg buds



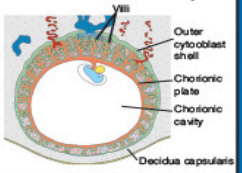
Day 30 Developing face



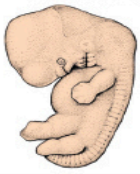
Day 31 Gut development



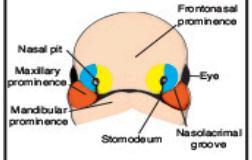
Day 32 Embryo in chorionic cavity



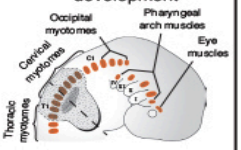
Day 36 Physiological umbilical hernia



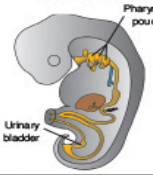
Day 37 Developing face



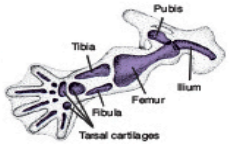
Day 38 Muscle development



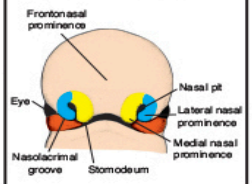
Day 39 Endodermal derivatives



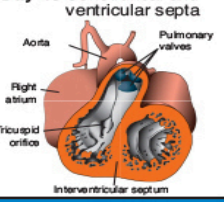
Day 43 Limb cartilages and digital rays



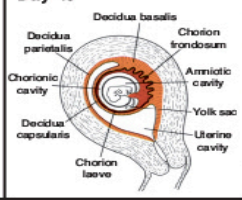
Day 44 Developing face



Day 45 Conotruncal and ventricular septa

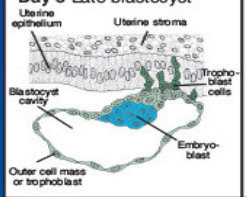


Day 46

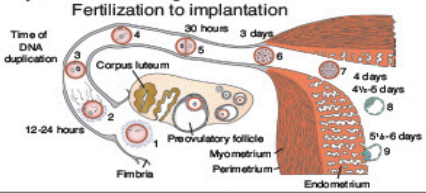


Embryonic Development in Days

Day 5 Late blastocyst

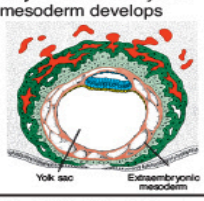


Day 6-7 Events during first week: Fertilization to implantation

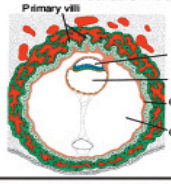


Development Week 1

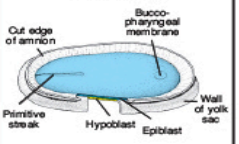
Day 12 Extraembryonic mesoderm develops



Day 13 Uteroplacental circulation begins

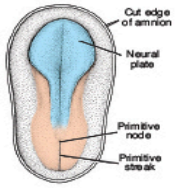


Day 14 Embryonic disc: dorsal view

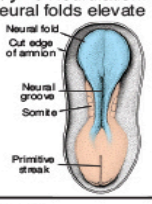


Development Week 2

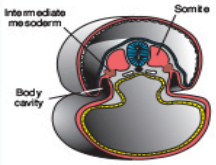
Day 19 CNS induction



Day 20 Neurulation: Neural folds elevate

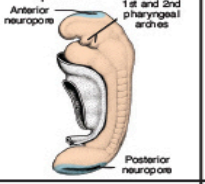


Day 21 Transverse section through somite region



Development Week 3

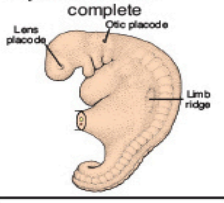
Day 26 Pharyngeal arches present



Day 27

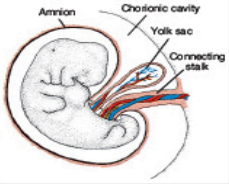
Approx. Age (Days)	No. of Somites
20	1-4
21	4-7
22	7-10
23	10-13
24	13-17
25	17-20
26	20-23
27	23-26
28	26-29
30	34-35

Day 28 Neurulation complete

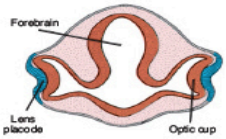


Development Week 4

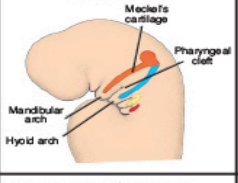
Day 33 Umbilical ring



Day 34 Optic cup and lens placode

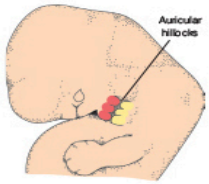


Day 35 Branchial arches and clefts

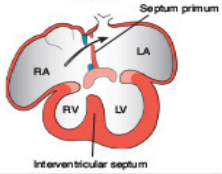


Development Week 5

Day 40 Auricular hillocks



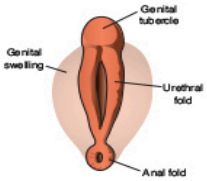
Day 41 Atrial septum formed



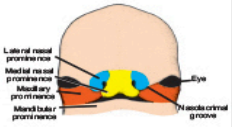
Day 42 Digit formation

Development Week 6

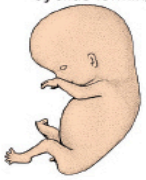
Day 47 External genitalia



Day 48 Facial prominences fused



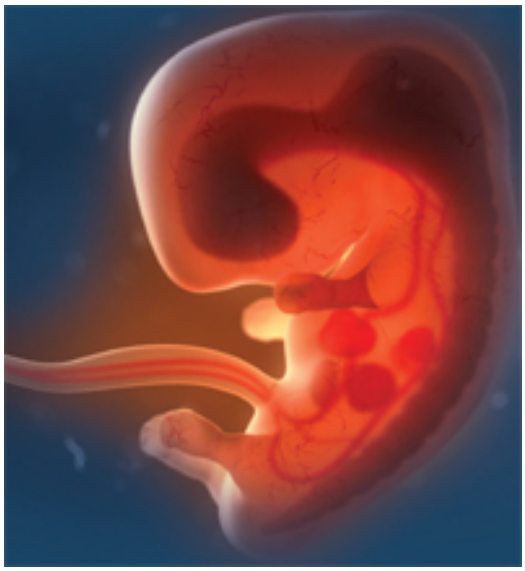
Day 49 Digits present, eyelids forming



Development Week 7

LANGMAN'S

Medical Embryology



Fifteenth Edition

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Scanning Electron Micrographs by
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Ultrasound Images by
Jan Byrne and Hytham Imseis

Wolters Kluwer

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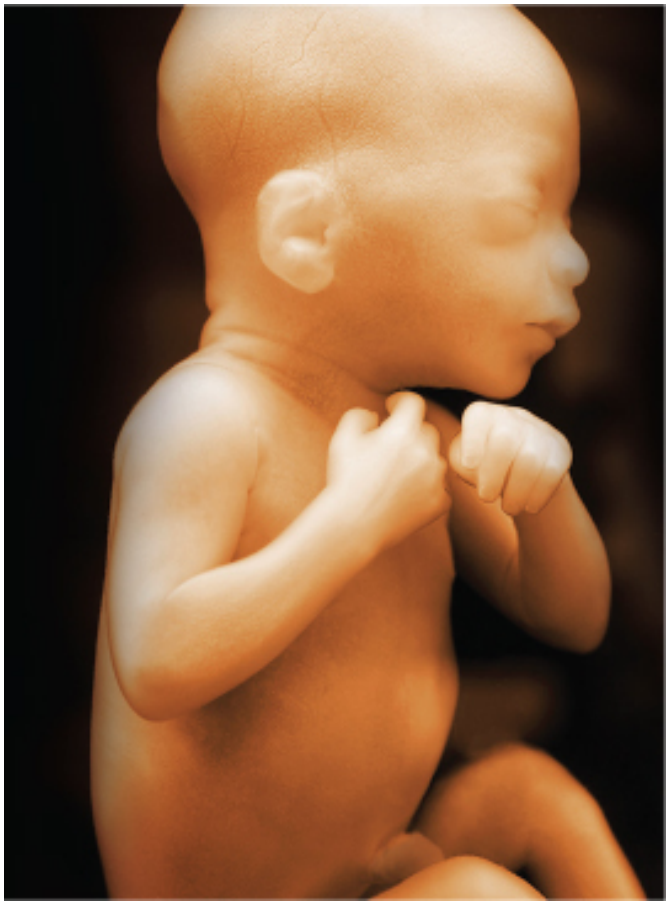
Dedication



For each and every child

Special thanks to:

Professor Neil Hanley* for providing new information concerning the embryologic origin of disorders of sex development (DSD). The incorporation of these concepts into this edition represents another example of the clinical relevance and importance of understanding the subjects of embryology and anatomy.

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Professor of Medicine
Faculty of Biology, Medicine, and Health
University of Manchester
United Kingdom

Preface

Every student will be affected by pregnancy, either: (1) directly from their own gestation, since adverse exposures during their mother's pregnancy can have lasting health care effects postnatally; (2) having their own child; (3) having a patient who is pregnant. In any case, pregnancy and childbirth are relevant to all of us, and, unfortunately, these processes often culminate in negative outcomes. For example, 50% of all embryos are spontaneously aborted. Furthermore, prematurity and birth defects are the leading causes of infant mortality and major contributors to disabilities. Fortunately, new strategies can improve pregnancy outcomes, and health care professionals have a major role to play in implementing these initiatives. However, a basic knowledge of embryology is essential to the success of these strategies, and with this knowledge, every health care professional can play a role in providing healthier babies.

To accomplish its goal of providing a basic understanding of embryology and its clinical relevance, *Langman's Medical Embryology* retains its unique approach of combining an economy of text with excellent diagrams and clinical images. It stresses the clinical importance of the subject by providing numerous clinical examples that result from abnormal embryologic events. The following pedagogic features and updates in the 15th edition help facilitate student learning.

Organization of Material: *Langman's Medical Embryology* is organized into two parts. The first provides an overview of early development from gametogenesis through the embryonic period. Also included in this section are chapters on placental and fetal development as well as prenatal diagnosis and birth defects. The second part of the text provides a description of the fundamental processes of embryogenesis for each organ system.

Clinical Correlates: In addition to describing normal events, each chapter contains clinical correlates that appear in highlighted boxes. This material is designed to demonstrate the clinical relevance of embryology and the importance of understanding key developmental events as a first step to improving birth outcomes and having healthier babies. Clinical pictures and case descriptions are used to provide this information, and this material has been increased and updated in this edition.

Genetics: Because of the increasingly important role of genetics and molecular biology in embryology and the study of birth defects, basic genetic and molecular principles are discussed. The first chapter provides an introduction to molecular processes, defines terms commonly used in genetics and molecular biology, and describes key pathways used in embryonic development. Then, throughout the text, major signaling pathways and genes that regulate embryologic development are identified and discussed.

Advances in the Field: The incorporation of information related to advances in the field of embryology have always been a focus for the book. Previously, new observations regarding the differentiation of somites and their contributions to development of the musculoskeletal system were added. New and important findings about laterality signaling and its role in cardiac development and the origin of many birth defects were also updated. In this edition, new concepts regarding the embryologic origins of disorders of sex development (DSD), organization of the autonomic nervous system (ANS), and timing for the origin of birth defects have been included.

Extensive Art Program: The artwork has always been designed to enhance understanding of the text and includes four-color line drawings, scanning electron micrographs, and clinical pictures. Once again, artwork has been added, especially to Chapter 18, to illustrate new concepts in development of the central nervous system, urogenital system, and other structures.

Summary: At the end of each chapter is a summary that serves as a concise review of the key points described in detail throughout the chapter. Key terms are highlighted and defined in these summaries.

Problems to Solve: Problems related to the key elements of each chapter are provided to assist the students in assessing their understanding of the material. Detailed answers are provided in an appendix at the back of the book.

Glossary: A glossary of key terms has been expanded and is located in the back of the book.

thePoint Web site: This site for students and instructors provides an interactive question bank of USMLE board-type questions. Teaching aids for instructors are also provided in the form of an image bank and a series of lectures on the major topics in embryology are presented in PowerPoint with accompanying notes.

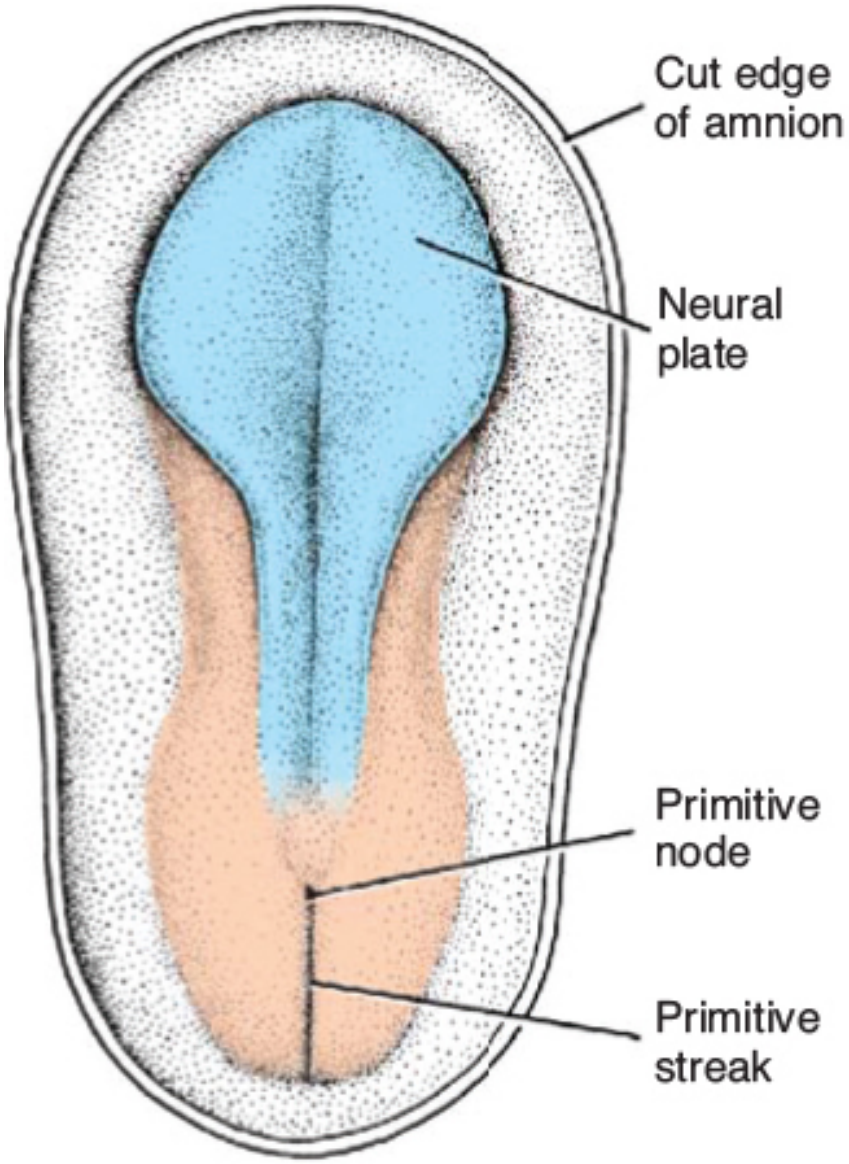
I hope you find this edition of *Langman's Medical Embryology* to be an excellent resource for learning embryology and its clinical significance. Together, the textbook and online resources are designed to provide a user-friendly and innovative approach to understanding the subject.

T. W. Sadler
Sheridan, MT

Contents

Part 3 · Appendix

Placode: **A local thickening in the embryonic ectoderm layer that develops into a sensory organ or ganglion.**



19 days

ODE TO A PLACODE

There once was a flat
sheet of cells
That were stumpy and
ugly as hell;
But one day they arose,
stood tall on their toes,
and declared they were
the best cells of all.

Presumptuously they
cried that their lineage
was high
and right proudly they
bragged of their codes;
But soon it was clear,
they weren't like the ear
and they were nixed in
their dreams as
placodes.

Semantics, they
screamed, please
maintain our dreams,
but their pleas were
unheeded and late;
And now to this day in
repast they must lay
as a misconstrued, flat
neural plate!

T. W. Sadler
Sheridan, MT

Embryology: Clinical Relevance and Historical Perspective

CLINICAL RELEVANCE

From a single cell to a baby in 9 months—a developmental process that represents an amazing integration of increasingly complex phenomena. The study of these phenomena is called **embryology**, and the field includes investigations of the molecular, cellular, and structural factors contributing to the formation of an organism. These studies are important because they provide knowledge essential for creating health care strategies for better reproductive outcomes. Thus, our increasingly greater understanding of embryology has resulted in new techniques for prenatal diagnoses and treatments; therapeutic procedures to circumvent problems with infertility; and mechanisms to prevent birth defects, the leading cause of infant mortality. These improvements in prenatal and reproductive health care are significant not only for their contributions to improved birth outcomes but also for their long-term effects postnatally. For example, both our cognitive capacity and our behavioral characteristics are affected by our prenatal experiences, and factors such as maternal smoking, nutrition, stress, diabetes, etc., play a role in our postnatal health. Furthermore, prenatal experiences, in combination with molecular and cellular factors, determine our potential to develop certain adult diseases, such as cancer and cardiovascular disease. Thus, our prenatal development produces many ramifications affecting our health for both the short and long terms, making the study of embryology and fetal development an important topic for all health care professionals. Also, with the exception of a few specialties, most physicians and health care workers will have an opportunity to interact with women of childbearing age, creating the potential for these providers to have a major impact on the outcome of developmental processes and their sequelae.

A BRIEF HISTORY OF EMBRYOLOGY

The process of progressing from a single cell through the period of establishing organ primordia (the first 8 weeks of human development) is called the period of **embryogenesis** (sometimes called the period of **organogenesis**); the period from that point on until birth is called the **fetal period**, a time when differentiation continues while the fetus grows and gains weight. Scientific approaches to study embryology have progressed over hundreds of years. Not surprisingly, anatomical approaches dominated early investigations. Observations were made, and these became more sophisticated with advances in optical equipment and dissection techniques. Comparative and evolutionary studies were part of this equation as scientists made comparisons among species and so began to understand the progression of developmental phenomena. Also investigated were offspring with birth defects, and these examples were compared to organisms with normal developmental patterns. The study of the embryologic origins and causes for these birth defects was called **teratology**.

In the 20th century, the field of experimental embryology blossomed. Numerous experiments were devised to trace cells during development to determine their cell lineages. These approaches included observations of transparent embryos from tunicates that contained pigmented cells which could be visualized through a microscope. Later, vital dyes were used to stain living cells to follow their fates. Still later in the 1960s, radioactive labels and autoradiographic techniques were employed. One of the first genetic markers also arose about this time with the creation of chick-quail chimeras. In this approach, quail cells, which have a unique pattern to their heterochromatin distribution around the nucleolus, were grafted into chick embryos at early stages of development. Later, host embryos were examined histologically, and the fates of the quail cells were determined. Permutations of this approach included development of antibodies specific to quail cell antigens that greatly assisted in the identification of these cells. Monitoring cell fates with these and other techniques provides valuable information about the origins of different organs and tissues.

Grafting experiments also provided the first insights into signaling between tissues. Examples of such experiments included grafting the primitive node from its normal position on the body axis to another and showing that this structure could induce a second germ disc. In another example, employing developing limb buds, it was shown that if a piece of tissue from the posterior axial border of one limb was grafted to the anterior border of a second limb, then digits on the host limb would be duplicated as the mirror image of each other. This posterior signaling region was called the zone of polarizing activity (ZPA), and it is now known that the signaling molecule is sonic hedgehog (SHH).

In 1961, the science of teratology became prominent because of the drug **thalidomide** that was given as an antinauseant and sedative to pregnant women. Unfortunately, the drug caused birth defects, including unique abnormalities of the limbs in which one or more limbs was absent (amelia) or was lacking the long bones such that only a hand or foot was attached to the torso (phocomelia). The association

between the drug and birth defects was recognized independently by two clinicians, W. Lenz and W. McBride, and showed that the conceptus was vulnerable to maternal factors that crossed the placenta. Soon, numerous animal models demonstrating an association between environmental factors, drugs, and genes provided further insights between developmental events and the origin of birth defects.

Today, molecular approaches have been added to the list of experimental paradigms used to study normal and abnormal development. Numerous means of identifying cells using reporter genes, fluorescent probes, and other marking techniques have improved our ability to map cell fates. Using other techniques to alter gene expression, such as knockout, knock-in, and antisense technologies, has created new ways to produce abnormal development and allowed the study of a single gene's function in specific tissues. Thus, the advent of molecular biology has advanced the field of embryology to the next level, and as we decipher the roles of individual genes and their interplay with environmental factors, our understanding of normal and abnormal developmental processes progresses.

Part 1

General Embryology

1 Introduction to Molecular Regulation and Signaling

INTRODUCTION

Molecular biology has opened the doors to new ways to study embryology and to enhance our understanding of normal and abnormal development. Sequencing the human genome, together with creating techniques to investigate gene regulation at many levels of complexity, has taken embryology to the next level. Thus, from the anatomical to the biochemical to the molecular level, the story of embryology has progressed, and each chapter has enhanced our knowledge.

Embryonic development is directed by **genomes** that contain all of the information required to make an individual. The information is encoded in **DNA** in sequences called **genes** that code for proteins. In turn, proteins regulate the expression of other genes and act as signal molecules to orchestrate development.

There are approximately 23,000 genes in the human genome, which represents only one-fifth of the number (100,000) predicted prior to completion of the Human Genome Project. Because of various levels of regulation, however, the number of proteins derived from these genes is closer to the originally predicted number of genes. What has been disproven is the one gene–one protein hypothesis; through a variety of mechanisms, a single gene may give rise to many proteins.

Gene expression can be regulated at several levels: (1) Different genes may be transcribed, (2) DNA transcribed from a gene may be selectively processed to regulate which RNAs reach the cytoplasm to become messenger RNAs (mRNAs), (3) mRNAs may be selectively translated, and (4) proteins made from the mRNAs may be differentially modified.

GENE TRANSCRIPTION

Genes are contained in a complex of DNA and proteins (mostly histones) called **chromatin**, and the basic unit of structure of chromatin is the **nucleosome** (Fig. 1.1). Each nucleosome is composed of an octamer of **histone proteins** and approximately 140 base pairs of DNA. Nucleosomes themselves are joined into clusters by the binding of DNA existing between nucleosomes (**linker DNA**) with other histone proteins (H1 histones; Fig. 1.1). Nucleosomes keep the DNA tightly coiled, such that it cannot be transcribed. In this inactive state, chromatin appears as beads of nucleosomes on a string of DNA and is referred to as **heterochromatin**. For

transcription to occur, this DNA must be uncoiled from the beads. In this uncoiled active state, chromatin is referred to as **euchromatin**.

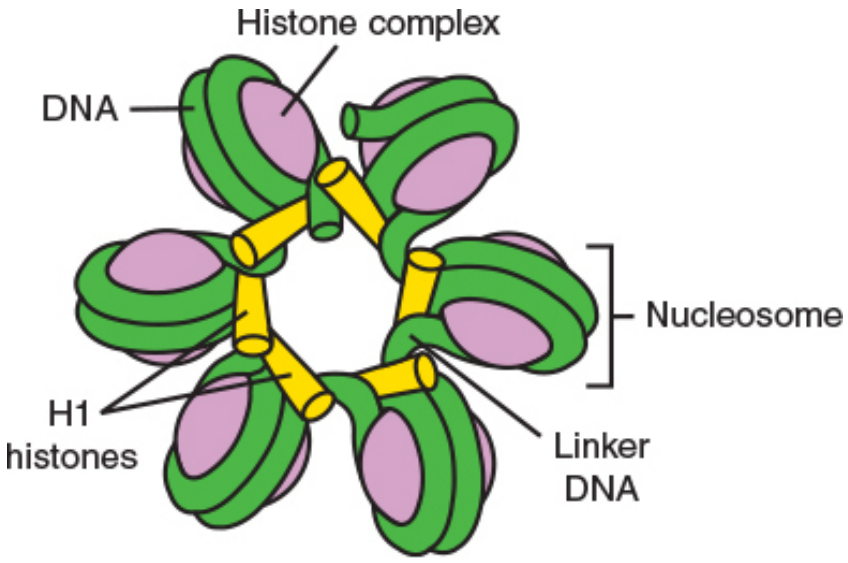


FIGURE 1.1 Drawing showing nucleosomes that form the basic unit of chromatin. Each nucleosome consists of an octamer of histone proteins and approximately 140 base pairs of DNA. Nucleosomes are joined into clusters by linker DNA and other histone proteins.

Genes reside within the DNA strand and contain regions called **exons**, which can be translated into proteins, and **introns**, which are interspersed between exons and are not transcribed into proteins (Fig. 1.2). In addition to exons and introns, a typical gene includes the following: a **promoter region** that binds **RNA polymerase** for the initiation of **transcription**; a **transcription initiation site**; a **translation initiation site** to designate the first amino acid in the protein; a **translation termination codon**; and a 3′ **untranslated region** that includes a sequence (the poly A addition site) that assists with stabilizing the mRNA, allows it to exit the nucleus, and permits it to be translated into protein (Fig. 1.2). By convention, the 5′ and the 3′ regions of a gene are specified in relation to the RNA transcribed from the gene. Thus, DNA is transcribed from the 5′ to the 3′ end, and the promoter region is upstream from the transcription initiation site (Fig. 1.2). The promoter region, where the RNA polymerase binds, usually contains the sequence TATA, and this site is called the **TATA box** (Fig. 1.2). In order to bind to this site, however, the polymerase requires additional proteins called **transcription factors** (Fig. 1.3). Transcription factors also have a specific **DNA-binding domain** plus a **transactivating domain** that activates or inhibits transcription of the gene whose promoter or enhancer it has bound. In combination with other proteins, transcription factors activate gene expression by causing the DNA nucleosome complex to unwind, by releasing the polymerase so that it can transcribe the DNA template, and by preventing new nucleosomes from forming.

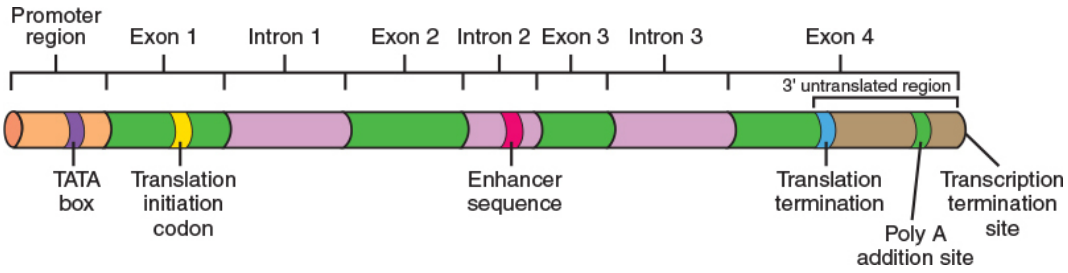


FIGURE 1.2 Drawing of a "typical" gene showing the promoter region containing the TATA box; exons that contain DNA sequences that are translated into proteins; introns; the transcription initiation site; the translation initiation site that designates the code for the first amino acid in a protein; and the 3′ untranslated region that includes the poly A addition site that participates in stabilizing the mRNA, allows it to exit the nucleus, and permits its translation into a protein.

Enhancers are regulatory elements of DNA that activate utilization of promoters to control the efficiency and the rate of transcription from the promoter. Enhancers can reside anywhere along the DNA strand and do not have to reside close to a promoter. Like promoters, enhancers bind transcription factors (through the transcription factor's transactivating domain) and are used to regulate the timing of a gene's expression and its cell-specific location. For example, separate enhancers in a gene can be used to direct the same gene to be expressed in different tissues. The *PAX6* transcription factor, which participates in pancreas, eye, and neural tube development, contains three separate enhancers, each of which regulates the gene's expression in the appropriate tissue. Enhancers act by altering chromatin to expose the promoter or by facilitating binding of the RNA polymerase. Sometimes, enhancers can inhibit transcription and are called **silencers**. This phenomenon allows a transcription factor to activate one gene while silencing another by binding to different enhancers. Thus, transcription factors themselves have a DNA-binding domain specific to a region of DNA plus a transactivating domain that binds to a promoter or an enhancer and activates or inhibits the gene regulated by these elements.

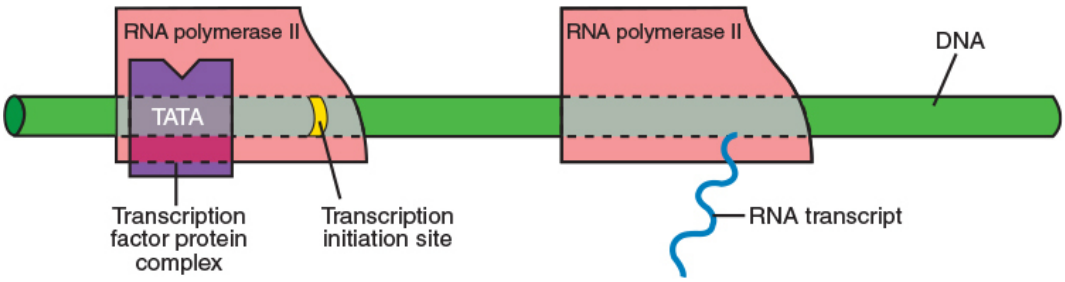


FIGURE 1.3 Drawing showing binding of RNA polymerase II to the TATA box site of the promoter region of a gene. This binding requires a complex of proteins plus an additional protein called a *transcription factor*. Transcription factors have their own specific DNA-binding domain and function to regulate gene expression.

DNA Methylation Represses Transcription

Methylation of cytosine bases in the promoter regions of genes represses transcription of those genes, thereby silencing some genes. For example, one of the X chromosomes in each cell of a female is inactivated (**X chromosome inactivation**) by this methylation mechanism. Similarly, genes in different types of cells are repressed by methylation, such that muscle cells make muscle proteins (their promoter DNA is mostly unmethylated) but not blood proteins (their DNA is highly methylated). In this manner, each cell can maintain its characteristic differentiated state. DNA methylation is also responsible for genomic **imprinting** in which only a gene inherited from the father or the mother is expressed, while the other gene is silenced. Approximately 40 to 60 human genes are imprinted, and their methylation patterns are established during spermatogenesis and oogenesis. Methylation silences DNA by inhibiting binding of transcription factors or by altering histone binding, resulting in stabilization of nucleosomes and tightly coiled DNA that cannot be transcribed. Factors that modulate gene expression without changing DNA sequences, like **methylation** and **histone** modification, are called **epigenetic modifiers**.

■ OTHER REGULATORS OF GENE EXPRESSION

The initial transcript of a gene is called **nuclear RNA (nRNA)** or sometimes **premessenger RNA**. nRNA is longer than mRNA because it contains introns that are removed **(spliced out)** as the nRNA moves from the nucleus to the cytoplasm. In fact, this splicing process provides a means for cells to produce different proteins from a single gene. For example, by removing different introns, exons are "spliced" in different patterns, a process called **alternative splicing** (Fig. 1.4). This process is carried out by **spliceosomes**, which are complexes of **small nuclear RNAs (snRNAs)** and proteins that recognize specific splice sites at the 5′ or the 3′ ends of the nRNA. Proteins derived from the same gene are called **splicing isoforms** (also called **splice variants** or **alternative splice forms**), and these afford the opportunity for different cells to use the same gene to make proteins specific for that cell type. For example, isoforms of the *WT1* gene have different functions in gonadal versus kidney development.

Even after a protein is made (translated), there may be **posttranslational modifications** that affect its function. For example, some proteins have to be cleaved to become active, or they might have to be phosphorylated. Others need to combine with other proteins, be released from sequestered sites, or be targeted to specific cell regions. Although only 23,000 genes exist, the many regulatory levels for synthesizing and activating proteins enable a potential number of proteins to be synthesized that is probably closer to five times the number of genes.

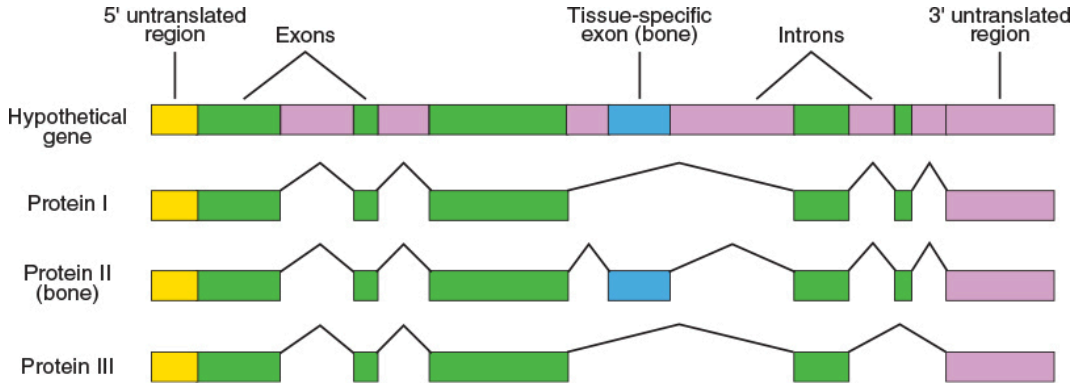


FIGURE 1.4 Drawing of a hypothetical gene illustrating the process of alternative splicing to form different proteins from the same gene. Spliceosomes recognize specific sites on the initial transcript of nRNA from a gene. Based on these sites, different introns are "spliced out" to create more than one protein from a single gene. Proteins derived from the same gene are called *splicing isoforms*.

INDUCTION AND ORGAN FORMATION

Organs are formed by interactions between cells and tissues. Most often, one group of cells or tissues causes another set of cells or tissues to change their fate, a process called **induction**. In each such interaction, one cell type or tissue is the **inducer** that produces a signal, and one is the **responder** to that signal. The capacity to respond to such a signal is called **competence**, and competence requires activation of the responding tissue by a **competence factor**. Many inductive interactions occur between epithelial and mesenchymal cells and are called **epithelial–mesenchymal interactions** (Fig. 1.5). Epithelial cells are joined together in tubes or sheets, whereas mesenchymal cells are fibroblastic in appearance and dispersed in extracellular matrices (Fig. 1.5). Examples of epithelial–mesenchymal interactions include the following: gut endoderm and surrounding mesenchyme to produce gut-derived organs, including the liver and pancreas; limb mesenchyme with overlying ectoderm (epithelium) to produce limb outgrowth and differentiation; and endoderm of the ureteric bud and mesenchyme from the metanephric blastema to produce nephrons in the kidney. Inductive interactions can also occur between two epithelial tissues, such as induction of the lens by epithelium of the optic cup. Although an initial signal by the inducer to the responder initiates the inductive event, **crosstalk** between the two tissues or cell types is essential for differentiation to continue (Fig. 1.5, *arrows*).

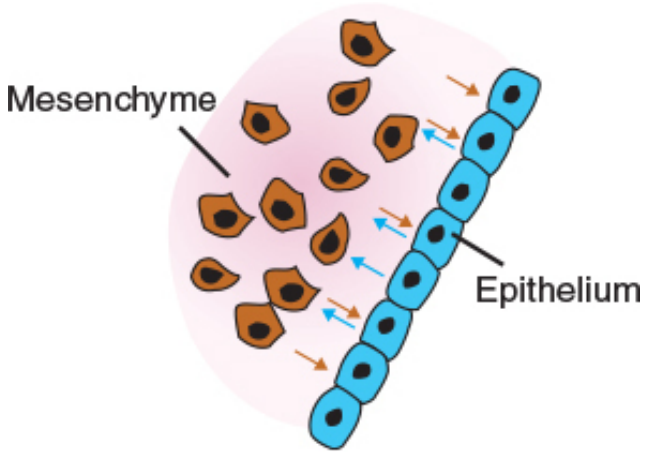


FIGURE 1.5 Drawing illustrating an epithelial–mesenchymal interaction. Following an initial signal from one tissue, a second tissue is induced to differentiate into a specific structure. The first tissue constitutes the inducer, and the second is the responder. Once the induction process is initiated, signals (*arrows*) are transmitted in both directions to complete the differentiation process.

CELL SIGNALING

Cell-to-cell signaling is essential for induction, for competency to respond, and for crosstalk between inducing and responding cells. These lines of communication are established by **paracrine interactions**, whereby proteins synthesized by one cell diffuse over short distances to interact with other cells, or by **juxtacrine interactions**, which do not involve diffusable proteins. The diffusable proteins responsible for paracrine signaling are called **paracrine factors** or **growth and differentiation factors (GDFs)**.

Signal Transduction Pathways

Paracrine Signaling

Paracrine factors act by **signal transduction pathways** either by activating a pathway directly or by blocking the activity of an inhibitor of a pathway (inhibiting an inhibitor, as is the case with hedgehog signaling). Signal transduction pathways include a **signaling molecule** (the **ligand**) and a **receptor** (Fig. 1.6). The receptor spans the cell membrane and has an **extracellular domain** (the **ligand-binding region**), a **transmembrane domain**, and a **cytoplasmic domain**. When a ligand binds its receptor, it induces a conformational change in the receptor that activates its cytoplasmic domain. Usually, the result of this activation is to confer enzymatic activity to the receptor, and most often, this activity is a **tyrosine kinase** that can **phosphorylate** other proteins using ATP as a substrate. In turn, phosphorylation activates these proteins to phosphorylate additional proteins, and thus, a cascade of protein interactions is established that ultimately activates a **transcription factor**. This

transcription factor then activates or inhibits gene expression. The pathways are numerous and complex and in some cases are characterized by one protein inhibiting another that in turn activates another protein (much like the situation with hedgehog signaling).

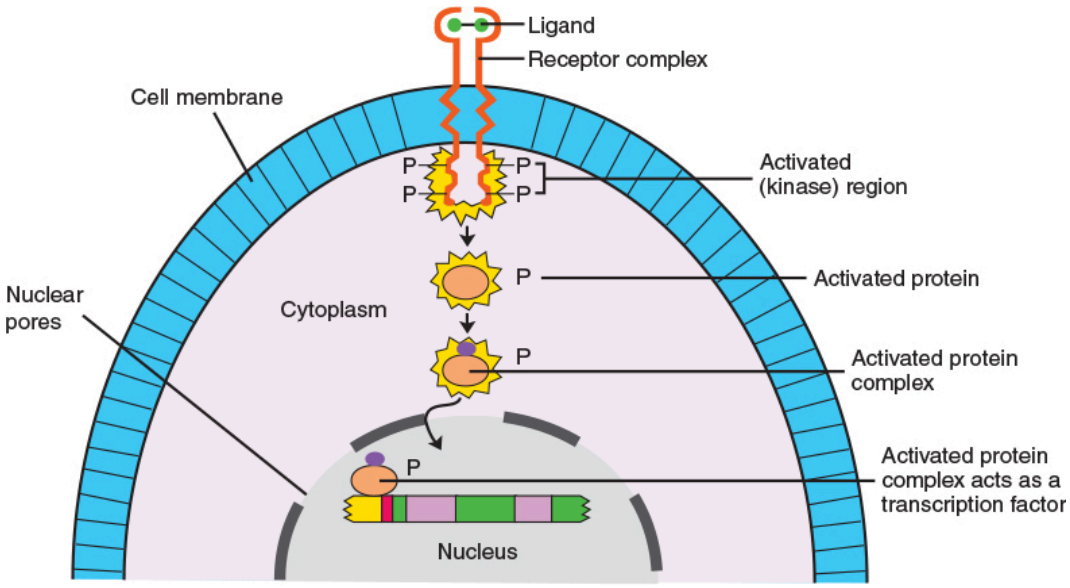


FIGURE 1.6 Drawing of a typical signal transduction pathway involving a ligand and its receptor. Activation of the receptor is conferred by binding to the ligand. Typically, the activation is enzymatic involving a tyrosine kinase, although other enzymes may be employed. Ultimately, kinase activity results in a phosphorylation cascade of several proteins that activates a transcription factor for regulating gene expression.

In some cases, gradients of paracrine factors regulate gene expression. Diffusible molecules that determine a cell's fate by establishing concentration gradients are called **morphogens**. In these examples, cells exposed to high concentrations of a morphogen express different genes that regulate the cell's fate than cells exposed to lower concentrations of the same morphogen. For example, varying concentrations of the morphogen retinoic acid regulate differentiation of different segments of the developing limb (see Chapter 12; p. 173).

Juxtacrine Signaling

Juxtacrine signaling is also mediated through signal transduction pathways but does not involve diffusable factors. Instead, there are three ways juxtacrine signaling occurs: (1) A protein on one cell surface interacts with a receptor on an adjacent cell in a process analogous to paracrine signaling (Fig. 1.6). The **Notch pathway** represents an example of this type of signaling (see "Key Signaling Pathways for Development," p. 9). (2) Ligands in the extracellular matrix secreted by one cell interact with their receptors on neighboring cells. The extracellular matrix is the milieu in which cells reside. This milieu consists of large molecules secreted by cells including **collagen**, **proteoglycans** (**chondroitin sulfates**, **hyaluronic acid**, etc.), and **glycoproteins**, such as **fibronectin** and **laminin**. These molecules provide a substrate for cells on

which they can anchor or migrate. For example, laminin and type IV collagen are components of the **basal lamina** for epithelial cell attachment, and fibronectin molecules form scaffolds for cell migration. Receptors that link extracellular molecules such as fibronectin and laminin to cells are called **integrins**. These receptors "integrate" matrix molecules with a cell's **cytoskeletal machinery** (e.g., **actin microfilaments**), thereby creating the ability to migrate along matrix scaffolding by using contractile proteins, such as **actin**. Also, integrins can induce gene expression and regulate differentiation as in the case of chondrocytes that must be linked to the matrix to form cartilage. (3) There is direct transmission of signals from one cell to another by **gap junctions**. These junctions occur as channels between cells through which small molecules and ions can pass. Such communication is important in tightly connected cells like epithelia of the gut and neural tube because they allow these cells to act in concert. The junctions themselves are made of **connexin proteins** that form a channel, and these channels are "connected" between adjacent cells.

It is important to note that there is a great amount of redundancy built into the process of signal transduction. For example, paracrine signaling molecules often have many family members such that other genes in the family may compensate for the loss of one of their counterparts. Thus, the loss of function of a signaling protein through a gene mutation does not necessarily result in abnormal development or death. In addition, there is crosstalk between pathways, such that they are intimately interconnected. These connections provide numerous additional sites to regulate signaling.

Paracrine Signaling Factors

There are a large number of **paracrine signaling factors** acting as ligands. Most are grouped into four families, and members of these families are used repeatedly to regulate development and differentiation of organ systems. Furthermore, the same factors regulate organ development throughout the animal kingdom, from *Drosophila* to humans. The four groups of paracrine factors include the **fibroblast growth factor (FGF), WNT**, **hedgehog**, and **transforming growth factor-β (TGF-β)** families. Each family of factors interacts with its own family of receptors, and these receptors are as important as the signal molecules themselves in determining the outcome of a signal.

Fibroblast Growth Factors

Originally named because they stimulate the growth of fibroblasts in culture, approximately two dozen **FGF** genes have now been identified, and they can produce hundreds of protein isoforms by altering their RNA splicing or their initiation codons. FGF proteins produced by these genes activate a collection of **tyrosine receptor kinases** called **fibroblast growth factor receptors (FGFRs)**. In turn, these receptors activate various signaling pathways. FGFs are particularly important for angiogenesis, axon growth, and mesoderm differentiation. Although there is redundancy in the family such that FGFs can sometimes substitute for one another, individual FGFs can be responsible for specific developmental events. For example, FGF8 is important for development of the limbs and parts of the brain.

Hedgehog Proteins

The ***hedgehog*** gene was named because it coded for a pattern of bristles on the leg of *Drosophila* that resembled the shape of a hedgehog. In mammals, there are three hedgehog genes: ***desert***, ***Indian***, and ***sonic hedgehog***. *Sonic hedgehog* (*SHH*) is involved in a multitude of developmental events (see "Key Signaling Pathways for Development," p. 9).

WNT Proteins

There are at least 15 different **WNT** genes that are related to the segment polarity gene, *wingless* in *Drosophila*. Their receptors are members of the **frizzled family** of proteins. WNT proteins are involved in regulating limb patterning, midbrain development, and some aspects of somite and urogenital differentiation among other actions.

The TGF-β Superfamily

The **TGF-β** superfamily has more than 30 members and includes the **TGF-βs**, the **bone morphogenetic proteins (BMPs)**, the **activin family**, the **müllerian inhibiting factor (MIF, anti-müllerian hormone)**, and others. The first member of the family, TGF-β1, was isolated from virally transformed cells. TGF-β members are important for extracellular matrix formation and epithelial branching that occurs in lung, kidney, and salivary gland development. The BMP family induces bone formation and is involved in regulating cell division, cell death (apoptosis), and cell migration among other functions.

Other Paracrine Signaling Molecules

Another group of paracrine signaling molecules important during development are neurotransmitters, including **serotonin**, **γ-amino butyric acid (GABA)**, **epinephrine**, and **norepinephrine**, that act as ligands and bind to receptors just as proteins do. These molecules are not just transmitters for neurons; they also provide important signals for embryologic development. For example, serotonin (5-HT) acts as a ligand for a large number of receptors, most of which are G protein–coupled receptors. Acting through these receptors, 5-HT regulates a variety of cellular functions, including cell proliferation and migration, and is important for establishing laterality, gastrulation, heart development, and other processes during early stages of differentiation. Norepinephrine also acts through receptors and appears to play a role in **apoptosis (programmed cell death)** in the interdigital spaces and in other cell types.

KEY SIGNALING PATHWAYS FOR DEVELOPMENT

Sonic Hedgehog: Master Gene for Embryogenesis

In the days before molecular biology, embryologists were convinced of the existence of a master signal that directed all of embryonic development. This signal would act as a **morphogen**, a secreted molecule that would establish concentration gradients and instruct cells in how to become different tissues and organs. Although we now know that there are a multitude of signaling molecules that coordinately regulate development, the protein **SHH** comes closest to being the master morphogen of them all. This protein is involved in development of the vasculature, left–right axis formation, midline, cerebellum, neural patterning, limbs, smooth muscle patterning, heart, gut, pharynx, lungs, pancreas, kidneys, bladder, hair follicles, teeth, thymocytes, inner ear, eyes, and taste buds: a veritable plethora of developmental events. Sonic signaling is via the pathway shown in Figure 1.7. The protein binds to its receptor, **Patched (Ptc)**, a protein that normally inhibits the receptor-like protein **Smoothened (Smo)**. When SHH binds to Ptc, Ptc activity is eliminated, the inhibition of Smo is removed, and Smo is activated to, ultimately, upregulate activity of the **glioma-associated oncogene (*GLI*)** family (1 to 3) of transcription factors that control expression of target genes. The specificity of *SHH* expression in different cell types is regulated by multiple enhancer elements acting independently to control *SHH* transcription in different cells and tissues.

The SHH protein has some unique characteristics, including the fact that after translation, it is cleaved and **cholesterol** is added to the C-terminus of its N-terminal domain. It is the addition of cholesterol that links SHH to the plasma membrane. Then, a palmitic acid moiety is added to the N-terminus and SHH becomes fully functional. Its release from the plasma membrane is produced by the transmembrane protein **Dispatched**. Cholesterol is essential for: (1) SHH transport out of the cell; (2) anchoring SHH to its receptor Patched; and (3) establishing gradients that allow SHH to act as a morphogen.

The Planar Cell Polarity: Convergent Extension Pathway

The **planar cell polarity (PCP) pathway** regulates the process of **convergent extension** whereby a tissue becomes longer and narrower (Fig. 1.8A). For example, during neural tube formation (neurulation), the neural plate narrows and elongates to form the neural groove between the neural folds. Similarly, during gastrulation, cells move medially and the embryonic axis elongates. Other examples of convergent

extension include elongation of the cardiac outflow tract and movement of the lateral body wall folds toward the midline. Convergent extension requires changes in cell shape together with cell movement and intercalation with other cells (Fig. 1.8A).

PCP refers to the reorganization of cells and cell sheets in the plane of a tissue, such as occurs during convergent extension. The principal PCP signaling pathway is the noncanonical **WNT** pathway, which includes the Wnt receptor **Frizzled (Fz)** and two other transmembrane proteins called **Celsr** and **Vangl** (Fig. 1.8B). These transmembrane proteins primarily target activation of *DISHEVELLED (DVL)*, either directly or through downstream effectors, such as Prickle (Pk) and Diego (Dgo). In turn, DVL regulates signaling via the Rho and Rac kinases to upregulate c-Jun N-terminal kinases (JNK) that control cytoskeletal changes and other downstream effectors including transcription factors. Mutations in many of these genes, including *FZ*, *CELSR*, *VANGL*, and *DVL*, have been shown to cause **neural tube defects** in mice and mutations in *VANGL* genes have been linked to these types of defects in humans.

The *Notch* Pathway

Notch transmembrane receptors bind to transmembrane ligands of the **DSL (Delta/Serrate/LAG-2)** family, which requires cell-to-cell contact (juxtacrine signaling) for signaling to occur. In mammals, there are four Notch family members and five transmembrane ligands (Jagged 1 and 2 and Delta 1 to 3). Binding of one of these proteins to a Notch receptor causes a conformational change in the Notch protein such that part of it on the cytoplasmic side of the membrane is cleaved. The pathway is very straightforward in that there are no second messengers involved. Thus, the cleaved portion of the protein enters the nucleus directly and binds to a DNA-binding protein that normally represses transcription of Notch target genes. Binding of Notch removes the inhibitory activity of the repressor and permits activation of downstream genes (Fig. 1.9).

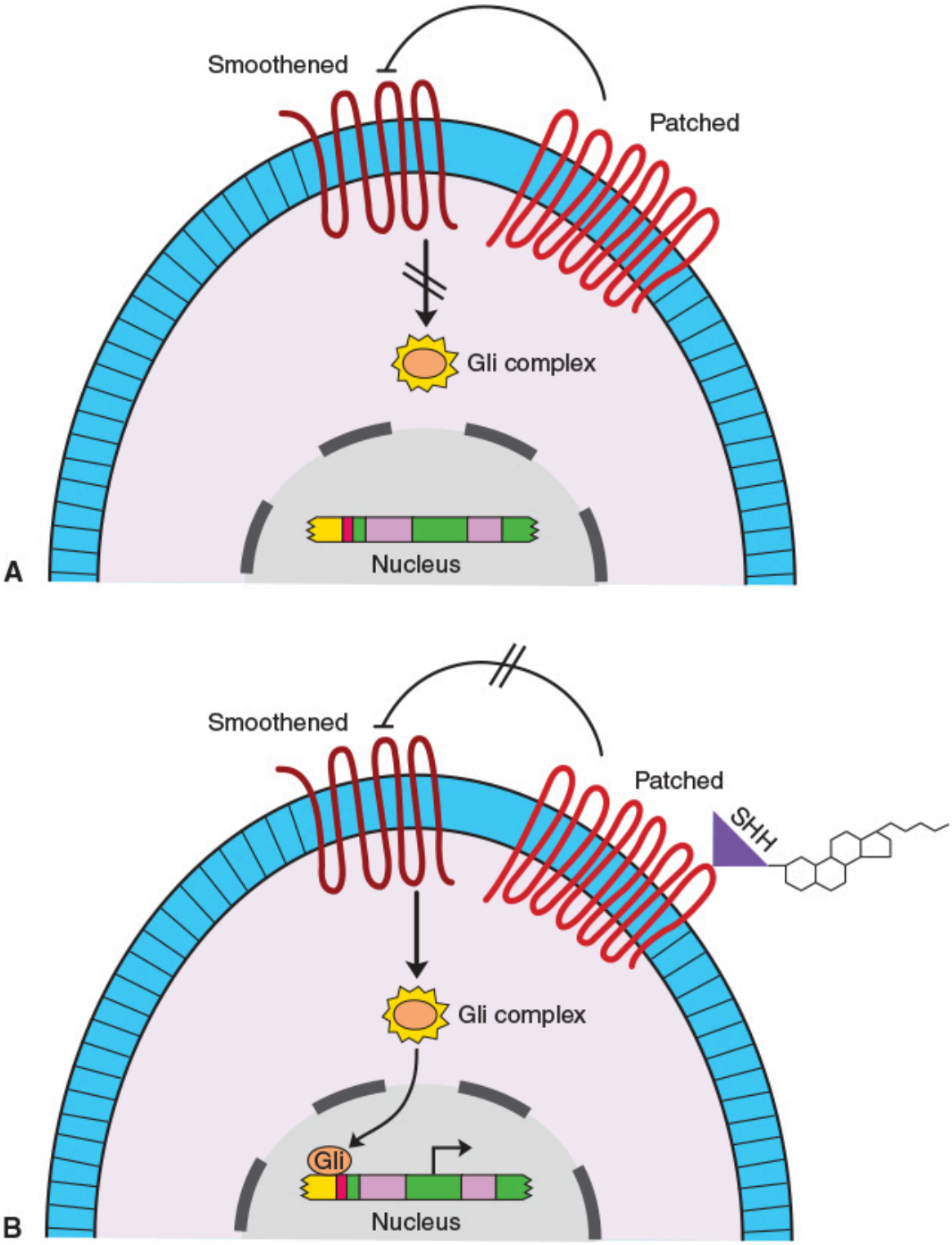


FIGURE 1.7 Drawings illustrating the sonic hedgehog (SHH) signaling pathway. **A.** Drawing of a cell showing Patched inhibition of Smoothened that blocks activation of the GLI proteins that normally transduce the SHH signal. **B.** Drawing showing SHH binding to its receptor Patched, that removes Patched inhibition of Smoothened. Activation of Smoothened then upregulates the GLI transcription factors that bind to DNA and control downstream effector genes in the SHH pathway.

Notch signaling is involved in cell proliferation, apoptosis, and epithelial to mesenchymal transitions. It is especially important in neuronal differentiation, blood vessel formation and specification (angiogenesis), somite segmentation, pancreatic β-cell development, B- and T-cell differentiation in the immune system, development of inner ear hair cells, and septation of the outflow tract of the heart. Mutations in *JAG1* or *NOTCH2* cause **Alagille syndrome,** characterized by cardiac outflow tract defects as well as skeletal, ocular, renal, and hepatic abnormalities. *JAG1* mutations have also been linked to cases of tetralogy of Fallot (a cardiac outflow tract defect).

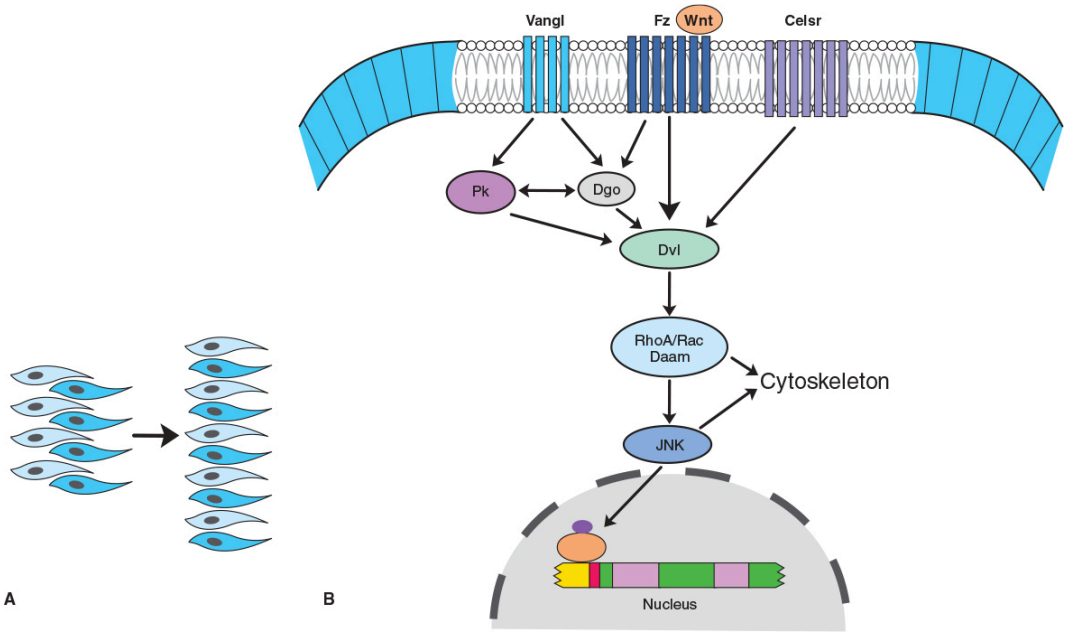


FIGURE 1.8 A. Drawing illustrating the process of convergent extension whereby cells intercalate with their neighbors to increase the long axis of a tissue, such as occurs during lengthening of the neural tube during neurulation. Convergent extension is dependent on the PCP pathway (the reorganization of cells and cell sheets in the plane of a tissue) that is regulated by the noncanonical *WNT* signaling pathway **(B)**. Wnt binds to its receptor Frizzled, which, together with two other transmembrane proteins *Celsr* and *Vangl*, activate *DISHEVELLED*. Dishevelled then acts through Rho and *Rac* kinases to upregulate c-Jun N-terminal kinases (*JNK*) that control cytoskeletal changes and downstream effectors, including transcription factors.

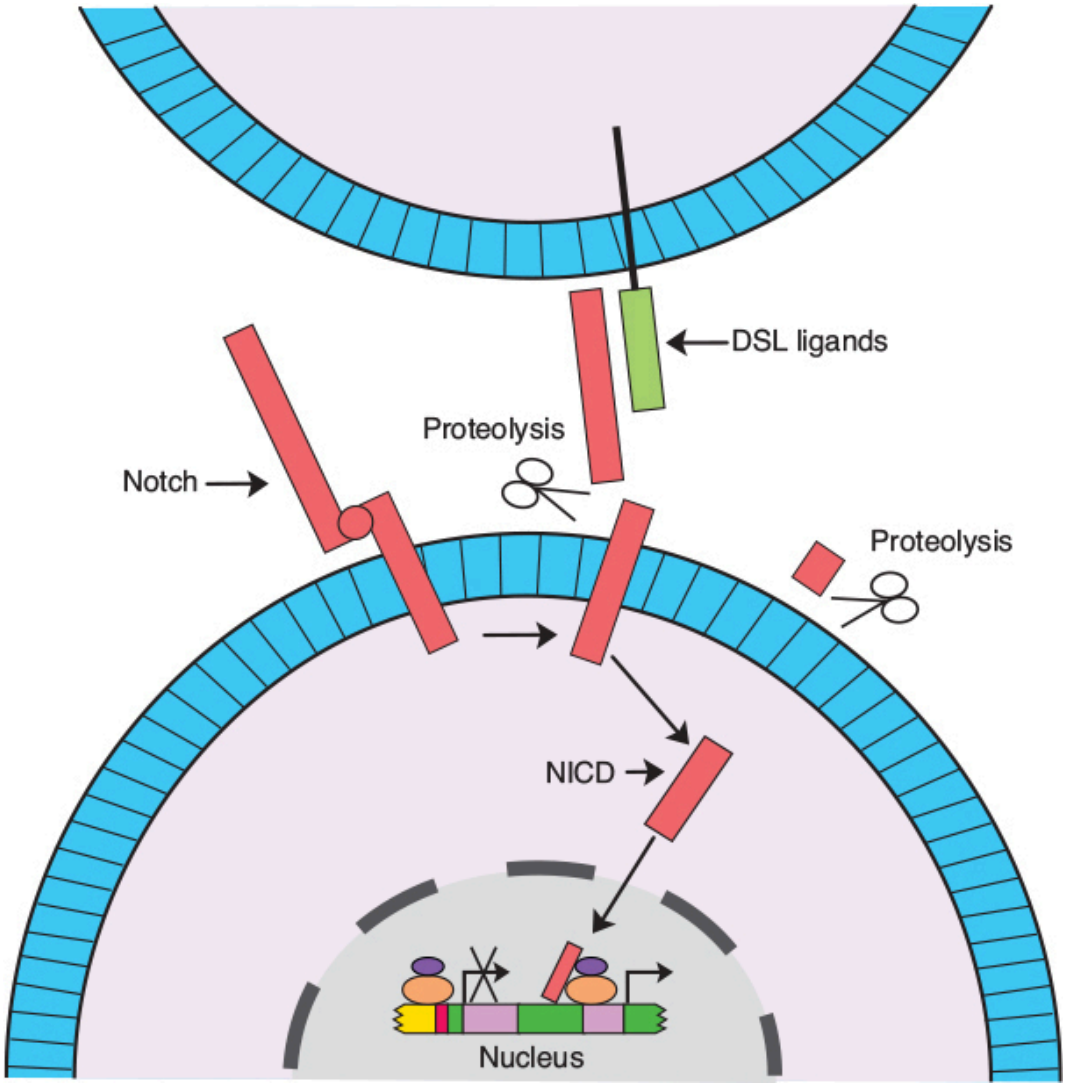


FIGURE 1.9 Drawing illustrating signaling via the *Notch* pathway. Notch receptors located on one cell bind a ligand from the *DSL* family (Jagged or Serrate) that are located on an adjacent cell (juxtacrine signaling), and this receptor–ligand interaction activates a proteolytic enzyme that cleaves the Notch protein to produce the activated membrane anchored Notch extracellular truncation (NEXT). NEXT is then cleaved by an intracellular secretase enzyme that results in the release of Notch intracellular domain (NICD) that represents the active signaling portion of the original Notch receptor. NICD translocates directly to the nucleus where it binds to transcription repressors and removes their inhibitory activity on downstream target genes of the Notch pathway.

SUMMARY

During the past century, embryology has progressed from an observational science to one involving sophisticated technologic and molecular advances. Together, observations and modern techniques provide a clearer understanding of the origins of normal and abnormal development and, in turn, suggest ways to prevent and treat birth

defects. In this regard, knowledge of gene function has created entire new approaches to the subject.

There are approximately 23,000 genes in the human **genome**, but these genes code for approximately 100,000 proteins. Genes are contained in a complex of DNA and proteins called **chromatin**, and its basic unit of structure is the nucleosome. Chromatin appears tightly coiled as beads of nucleosomes on a string and is called **heterochromatin**. For transcription to occur, DNA must be uncoiled from the beads as **euchromatin**. Genes reside within strands of DNA and contain regions that can be translated into proteins, called **exons**, and untranslatable regions, called **introns**. A typical gene also contains a **promoter region** that binds **RNA polymerase** for the initiation of transcription; a **transcription initiation site**, to designate the first amino acid in the protein; a **translation termination codon**; and a 3′ untranslated region that includes a sequence (the poly A addition site) that assists with stabilization of the mRNA. The RNA polymerase binds to the promoter region that usually contains the sequence TATA, the **TATA box**. Binding requires additional proteins called **transcription factors**. Methylation of cytosine bases in the promoter region silences genes and prevents transcription. This process is responsible for **X chromosome inactivation** whereby the expression of genes on one of the X chromosomes in females is silenced and also for genomic **imprinting** in which either a paternal or a maternal gene's expression is repressed.

Different proteins can be produced from a single gene by the process of **alternative splicing** that removes different introns using **spliceosomes**. Proteins derived in this manner are called **splicing isoforms** or **splice variants**. Also, proteins may be altered by **posttranslational modifications**, such as phosphorylation or cleavage.

Induction is the process whereby one group of cells or tissues (the **inducer**) causes another group (the **responder**) to change their fate. The capacity to respond is called **competence** and must be conferred by a **competence factor**. Many inductive phenomena involve **epithelial–mesenchymal interactions**.

Signal transduction pathways include a signaling molecule (the **ligand**) and a **receptor**. The receptor usually spans the cell membrane and is activated by binding with its specific ligand. Activation usually involves the capacity to phosphorylate other proteins, most often as a **tyrosine kinase.** This activation establishes a cascade of enzyme activity among proteins that ultimately activates a transcription factor for initiation of gene expression.

Cell-to-cell signaling may be **paracrine**, involving **diffusable factors**, or **juxtacrine**, involving a variety of **nondiffusable factors**. Proteins responsible for paracrine signaling are called **paracrine factors**. There are four major families of these factors: **FGFs**, **WNTs**, **hedgehogs**, and **TGF-βs**. In addition to proteins, **neurotransmitters**, such as **serotonin (5-HT)** and **norepinephrine**, also act through paracrine signaling, serving as ligands and binding to receptors to produce specific cellular responses. Juxtacrine factors may include products of the extracellular matrix, ligands bound to a cell's surface, and direct cell-to-cell communications.

There are many cell signaling pathways important for development, but two key pathways involve the protein **SHH** and the **noncanonical *WNT* pathway**, better known

as the **PCP pathway** (**planar cell polarity**) that regulates **convergent extension**. ***SHH*** is almost a **master gene**, and when this gene's protein product binds to its receptor **patched**, it removes patched inhibition of **smoothened**. Once activated, smoothened causes upregulation of the ***GLI*** family of transcription factors that control downstream signaling by SHH. SHH is a diffusible factor that acts as a **morphogen** by establishing concentration gradients that regulate cell fates. Cholesterol is attached to SHH and is responsible for: (1) SHH transport out of cells; (2) establishing concentration gradients of SHH; and (3) binding of SHH to its receptor Patched. SHH signaling is involved in many developmental events, including establishing the midline and left–right asymmetry and in patterning many different organs.

The **PCP** regulates movements of cells and sheets of cells in the plane of a tissue, such that the cells intercalate with other cells in such a way that the tissue elongates, a process called **convergent extension**. These types of cell movements are responsible for lengthening the embryo and the neural tube during gastrulation and neurulation, respectively. Several genes are involved in regulating this process, including **WNT** and its receptor **FRIZZLED**, ***CELSR***, and ***VANGL,*** which code for transmembrane proteins, and ***DISHEVELLED,*** which codes for a protein that acts through Rho and Rac kinases to affect the cytoskeleton and other genes regulating cell movements. Mutations in these genes cause neural tube defects in mice, and those involving VANGL have been linked to these defects in humans.

Problems to Solve

1. What is meant by "competence to respond" as part of the process of induction? What tissues are most often involved in induction? Give two examples.
2. Under normal conditions, FGFs and their receptors (FGFRs) are responsible for growth of the skull and development of the cranial sutures. How might these signaling pathways be disrupted? Do these pathways involve paracrine or juxtacrine signaling? Can you think of a way that loss of expression of one FGF might be circumvented?

2 Gametogenesis: Conversion of Germ Cells into Male and Female Gametes

PRIMORDIAL GERM CELLS

Development begins with fertilization, the process by which the male gamete, the **sperm**, and the female gamete, the **oocyte**, unite to give rise to a **zygote**. Gametes are derived from **primordial germ cells (PGCs)** that are formed in the epiblast during the second week, move through the primitive streak during gastrulation, and migrate to the wall of the yolk sac (Fig. 2.1). During the fourth week, these cells begin to migrate from the yolk sac toward the developing gonads, where they arrive by the end of the fifth week. Mitotic divisions increase their number during their migration and also when they arrive in the gonad. In preparation for fertilization, germ cells undergo **gametogenesis**, which includes meiosis, to reduce the number of chromosomes, and **cytodifferentiation** to complete their maturation.

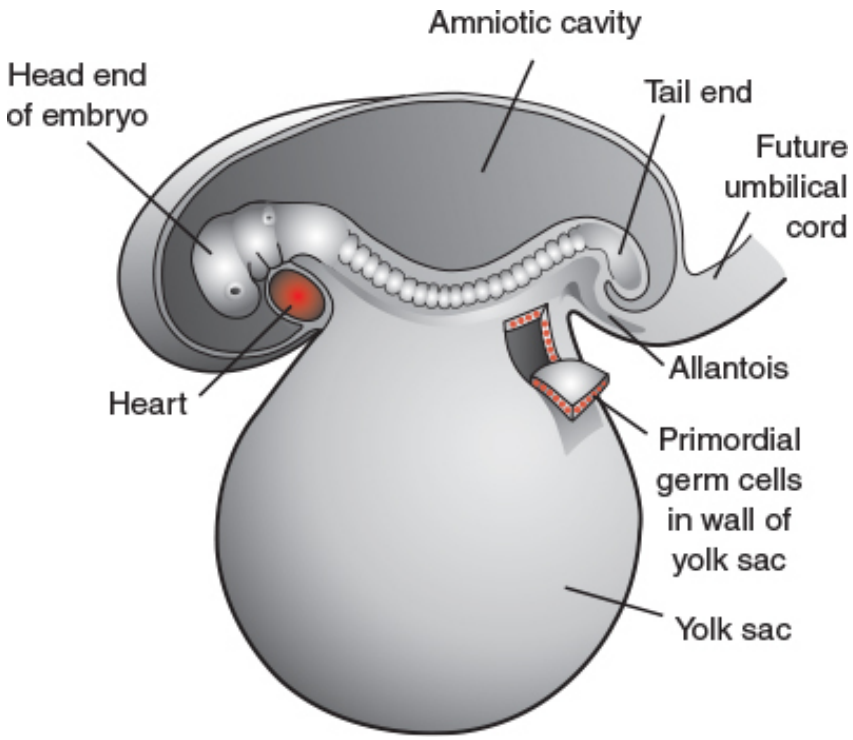


FIGURE 2.1 An embryo at the end of the third week, showing the position of primordial germ cells (PGCs) in the wall of the yolk sac, close to the attachment of the future umbilical cord. From this location, these cells migrate to the developing gonad.

Clinical Correlates

Primordial Germ Cells and Teratomas

Teratomas are tumors of disputed origin that often contain a variety of tissues, such as bone, hair, muscle, gut epithelia, and others. It is thought that these tumors arise from pluripotent stem cells that can differentiate into any of the three germ layers or their derivatives. Some evidence suggests that primordial germ cells (PGCs) that have strayed from their normal migratory paths could be responsible for some of these tumors (Fig. 2.2). Another source may be epiblast cells that give rise to all three germ layers during gastrulation (see p. 66 and Fig. 5.9, p. 67).

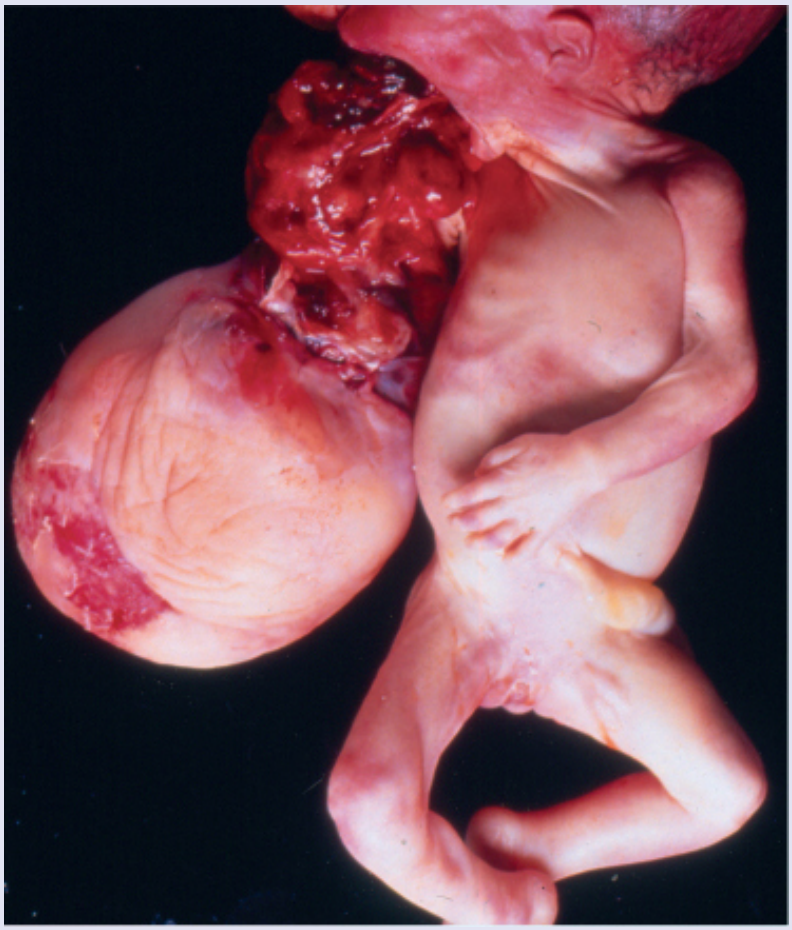


FIGURE 2.2 Oropharyngeal teratoma. These tumors may arise from PGCs or from epiblast cells (see Chapter 5), both of which are pluripotent. Tissues within the tumors include derivatives of all three germ layers and may include gut, bone, skin, teeth, and so forth.

THE CHROMOSOME THEORY OF INHERITANCE

Traits of a new individual are determined by specific genes on chromosomes inherited from the father and the mother. Humans have approximately 23,000 genes on 46 chromosomes. Genes on the same chromosome tend to be inherited together and so are known as **linked genes**. In somatic cells, chromosomes appear as 23 **homologous** pairs to form the **diploid** number of 46 (2n). There are 22 pairs of matching chromosomes, the **autosomes**, and one pair of **sex chromosomes**. If the sex pair is XX, the individual is genetically female; if the pair is XY, the individual is genetically male. One chromosome of each pair is derived from the maternal gamete, the **oocyte**, and one from the paternal gamete, the **sperm**. Thus, each gamete contains a **haploid** number of 23 chromosomes, and the union of the gametes at **fertilization** restores the diploid number of 46 (2n).

Mitosis

Mitosis is the process whereby one cell divides, giving rise to two daughter cells that are genetically identical to the parent cell (Fig. 2.3; Table 2.1). Prior to mitosis, most cells spend their time in **interphase**, a time when cells accumulate nutrients and duplicate their **DNA**. Cells in a proliferating population alternate between interphase and mitosis as they pass through the **cell cycle.** The interphase portion of the cycle is divided into three parts: **G1, S,** and **G2.** G1 occurs between mitosis and DNA replication and is the stage when protein synthesis and normal cellular function occurs. S is the stage when DNA is replicated; duplicated chromosomes that are formed at this stage are called **sister chromatids.** G2 is the stage when some DNA repair occurs and the cell becomes ready for mitosis. With the onset of mitosis, chromosomes begin to coil, contract, and condense; these events mark the beginning of **prophase**. Each chromosome consists of two parallel subunits, sister chromatids, that are joined at a narrow region common to both called the **centromere**. Throughout prophase, chromosomes continue to condense, shorten, and thicken (Fig. 2.3A), but only at prometaphase do the chromatids become distinguishable (Fig. 2.3B). Also, at this time the nuclear envelope disappears and **spindle fibers** run like cables between **centromeres** and **centrioles** located at opposite poles of the cell (Fig. 2.3C). During **metaphase,** chromosomes line up in the equatorial plane, and their doubled structure is clearly visible (Fig. 2.3C). Each is attached by **microtubules** extending from the centromere to the centriole, forming the **mitotic spindle**. Soon, the centromere of each chromosome divides, marking the beginning of **anaphase** followed by migration of chromatids to opposite poles of the spindle. Finally, during **telophase**, chromosomes uncoil and lengthen, the nuclear envelope reforms, and the cytoplasm divides (Fig. 2.3D–F). Each daughter cell receives half of all doubled chromosome material and thus maintains the same number of chromosomes as the mother cell.

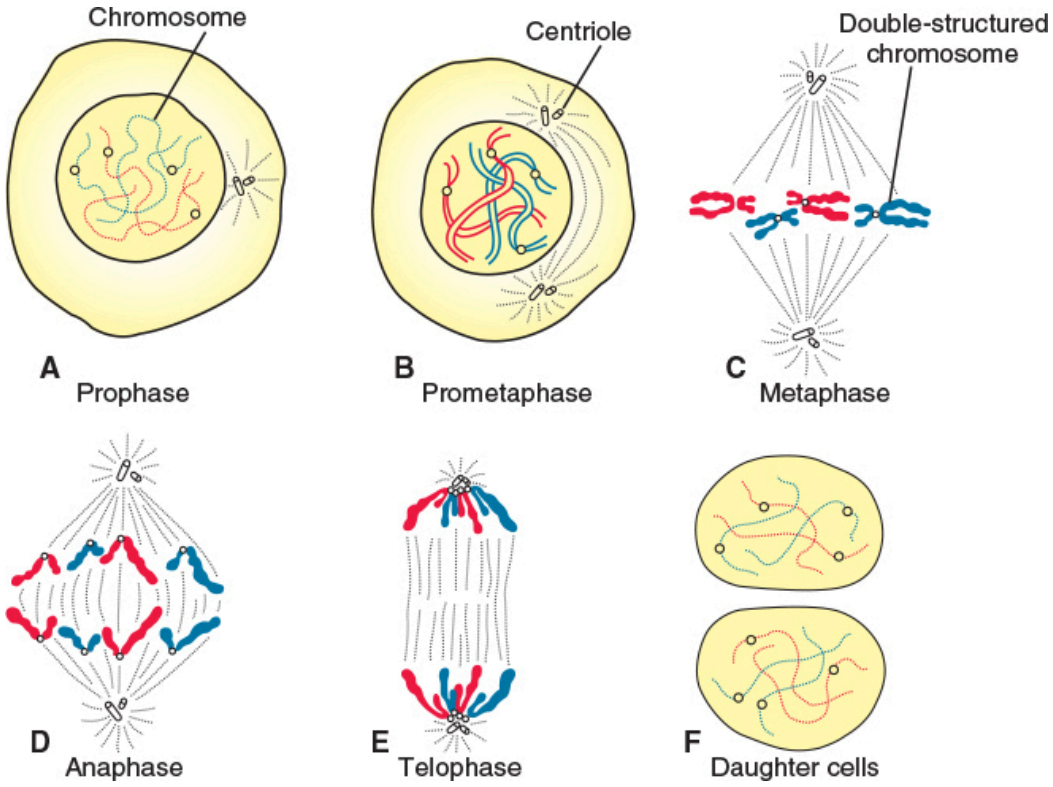


FIGURE 2.3 Various stages of mitosis. In prophase, chromosomes are visible as slender threads. Doubled chromatids become clearly visible as individual units during metaphase. At no time during division do members of a chromosome pair unite. *Blue*, paternal chromosomes; *red*, maternal chromosomes.

TABLE 2.1 Mitosis: Key Events and Chromosome Number

Stage	Cellular Events	Chromosome Number
Interphase	Resting stage until DNA replication occurs	Diploid
Prophase	Chromosomes condense Sister chromatids of each chromosome attach to centromeres	Diploid (2× DNA)
Prometaphase	Nuclear envelope degenerates Spindle apparatus forms, connecting centromeres to centrioles	Diploid (2× DNA)
Metaphase	Chromosomes align on cell's equator Centromeres are duplicated	Diploid (2× DNA)
Anaphase	Centromeres split Chromatids pulled to opposite poles of the cell by contraction	Tetraploid

	of spindle fibers	
Telophase	Nuclear envelopes form Chromosomes uncoil Cytokinesis occurs	Diploid

Meiosis

Meiosis is the cell division that takes place in the **germ cells** to generate male and female gametes, sperm and egg cells, respectively. Meiosis requires two cell divisions, **meiosis I** and **meiosis II**, to reduce the number of chromosomes to the haploid number of 23 (Fig. 2.4). As in mitosis, male and female germ cells (**spermatocytes** and **primary oocytes**) at the beginning of meiosis I replicate their DNA so that each of the 46 chromosomes is duplicated into sister chromatids. In contrast to mitosis, however, **homologous chromosomes** then align themselves in **pairs**, a process called **synapsis**. The pairing is exact and point for point except for the XY combination. Homologous pairs then separate into two daughter cells, thereby reducing the chromosome number from diploid to haploid. Shortly thereafter, meiosis II separates sister chromatids. Each gamete then contains 23 chromosomes (Fig. 2.4; Table 2.2).

Crossover

Crossovers, critical events in meiosis I, are the **interchange of chromatid segments** between paired homologous chromosomes (Fig. 2.4C). Segments of chromatids break and are exchanged as homologous chromosomes separate. As separation occurs, points of interchange are temporarily united and form an X-like structure, a **chiasma** (Fig. 2.4C). The approximately 30 to 40 crossovers (one or two per chromosome) with each meiotic I division are most frequent between genes that are far apart on a chromosome.

As a result of meiotic divisions:

- **Genetic variability** is enhanced through
  - Crossover, which redistributes genetic material
  - Random distribution of homologous chromosomes to the daughter cells
- Each germ cell contains a haploid number of chromosomes so that at fertilization, the diploid number of 46 is restored.

Polar Bodies

Also during meiosis, one primary oocyte gives rise to four daughter cells, each with 22 plus 1 X chromosomes (Fig. 2.5A). Only one of these develops into a mature gamete, the oocyte; the other three, the **polar bodies**, receive little cytoplasm and degenerate during subsequent development. Similarly, one primary spermatocyte gives rise to four daughter cells, two with 22 plus 1 X chromosomes and two with 22 plus 1 Y chromosomes (Fig. 2.5B). In contrast to oocyte formation, all four develop into mature gametes.

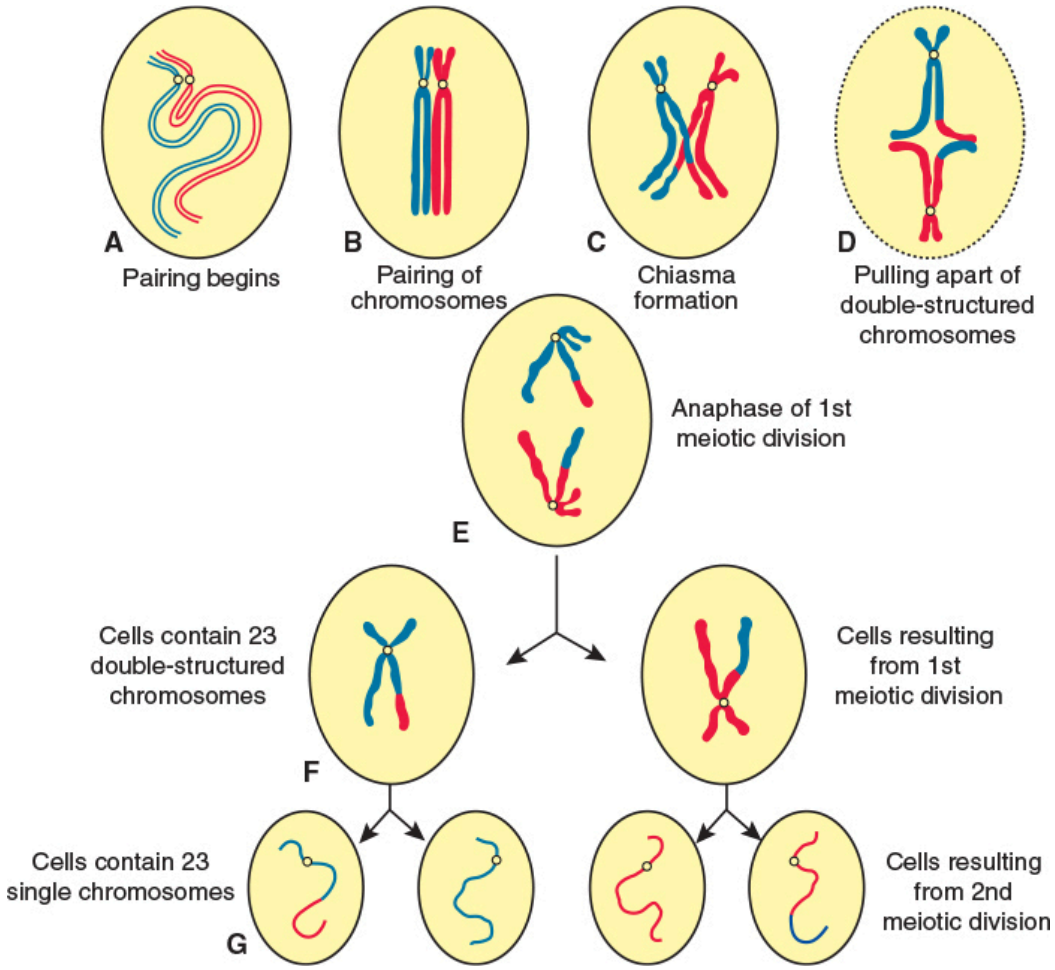


FIGURE 2.4 First and second meiotic divisions. **A.** Homologous chromosomes approach each other. **B.** Homologous chromosomes pair, and each member of the pair consists of two chromatids. **C.** Intimately paired homologous chromosomes interchange chromatid fragments (crossover). Note the chiasma. **D.** Double-structured chromosomes pull apart. **E.** Anaphase of the first meiotic division. **F,G.** During the second meiotic division, the double-structured chromosomes split at the centromere. At completion of division, chromosomes in each of the four daughter cells are different from each other.

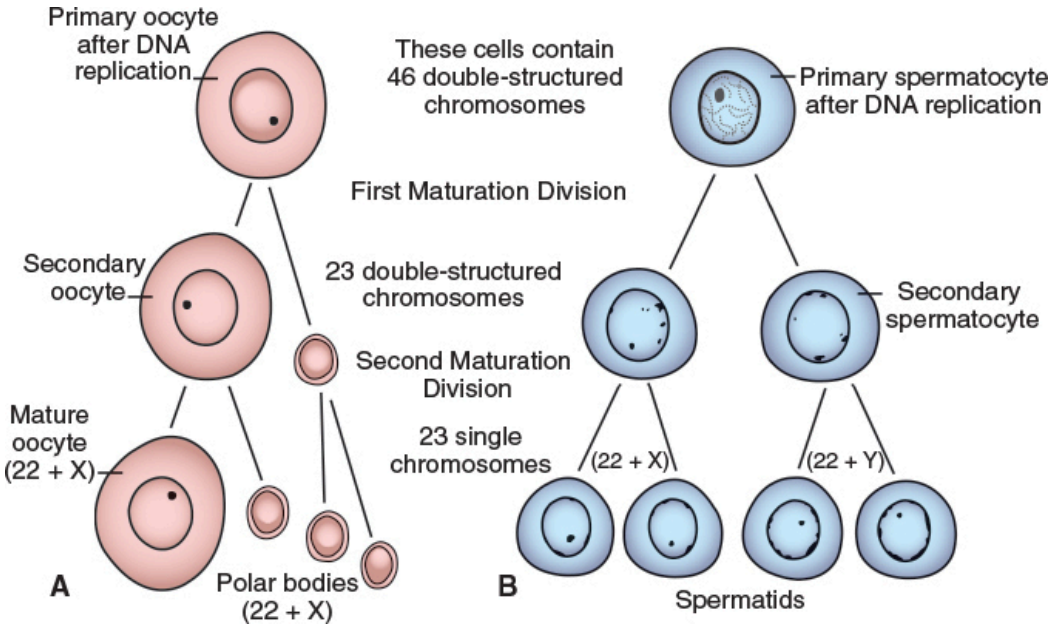


FIGURE 2.5 Events occurring during the first and second maturation divisions. **A.** The primitive female germ cell (primary oocyte) produces only one mature gamete, the mature oocyte. **B.** The primitive male germ cell (primary spermatocyte) produces four spermatids, all of which develop into spermatozoa.

TABLE 2.2 Meiosis: Key Events and Chromosome Number

Stage	Cellular Events	Chromosome Number
	Meiosis I	
Interphase I	DNA replication	Diploid
Prophase I	Chromosomes condense Homologous chromosomes pair at their centromeres Chiasmata form and crossovers occur	Diploid
Metaphase I	Homologous pairs align in the equatorial plane Spindle fibers appear	Diploid
Anaphase I	Centromeres do not replicate Homologous chromosomes pulled by spindle fibers to opposite poles of the cell	Diploid
Telophase I	Chromosomes uncoil Nuclear envelopes form Cytokinesis occurs	Haploid
	Meiosis II	
Interphase II	No duplication of DNA	Haploid

	Very short in duration	
Prophase II	Chromosomes condense Nuclear envelope degenerates Spindle fibers form	Haploid
Metaphase II	Chromosomes align on the equatorial plane	Haploid
Anaphase II	Centromeres split Spindle fibers contract pulling sister chromatids to opposite poles of the cell	Haploid
Telophase II	Nuclear envelopes form Chromosomes uncoil Cytokinesis occurs	Haploid

Clinical Correlates

Birth Defects and Spontaneous Abortions: Chromosomal and Genetic Factors

Chromosomal abnormalities, which may be **numerical** or **structural**, are important causes of birth defects and spontaneous abortions. It is estimated that 50% of conceptions end in spontaneous abortion and that 50% of these abortuses have major chromosomal abnormalities. Thus, approximately 25% of conceptuses have a major chromosomal defect. The most common chromosomal abnormalities in abortuses are 45,X (Turner syndrome), triploidy, and trisomy 16. Together, chromosome abnormalities and **gene mutations** account for approximately 30% of the total number of birth defects.

Numerical Abnormalities

The normal human somatic cell contains 46 chromosomes; the normal gamete contains 23. Normal somatic cells are **diploid**, or **2*n***; normal gametes are **haploid**, or ***n***. **Euploid** refers to any exact multiple of n (e.g., diploid or triploid). **Aneuploid** refers to any chromosome number that is not euploid; it is usually applied when an extra chromosome is present (**trisomy**) or when one is missing (**monosomy**). Abnormalities in chromosome number may originate during meiotic or mitotic divisions. In **meiosis**, two members of a pair of homologous chromosomes normally separate during the first meiotic division so that each daughter cell receives one member of each pair (Fig. 2.6A). Sometimes, however, separation does not occur (**nondisjunction**), and both members of a pair move into one cell (Fig. 2.6B,C). As a result of nondisjunction of the chromosomes, one cell receives 24 chromosomes and the other receives 22 instead of the normal 23. When, at fertilization, a gamete having 23 chromosomes fuses with a gamete having 24 or 22 chromosomes, the result is an individual with either 47 chromosomes (trisomy) or 45 chromosomes (monosomy). Nondisjunction, which occurs during either the first or the second meiotic division of the germ cells, may involve the autosomes or sex chromosomes. In women, the incidence of chromosomal abnormalities, including nondisjunction, increases with age, especially at 35 years and older.

Sometimes, chromosomes break, and pieces of one chromosome attach to another. Such **translocations** may be **balanced**, in which case breakage and reunion occur between two chromosomes, but no critical genetic material is lost and individuals are normal; or they may be **unbalanced**, in which case part of one chromosome is lost, and an altered phenotype is produced. For example, unbalanced translocations between the long arms of chromosomes 14 and 21 during meiosis I or II produce gametes with an extra copy of chromosome 21, one of the causes of Down syndrome (Fig. 2.7). Translocations are particularly common between chromosomes 13, 14, 15, 21, and 22 because they cluster during meiosis.

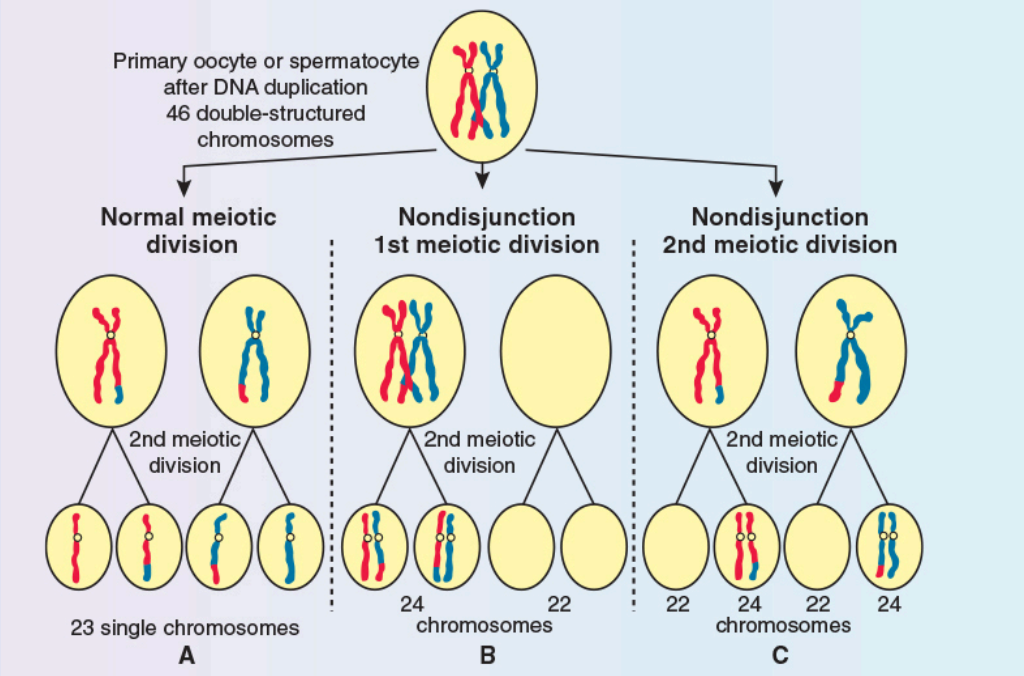


FIGURE 2.6 **A.** Normal maturation divisions. **B.** Nondisjunction in the first meiotic division. **C.** Nondisjunction in the second meiotic division.

TRISOMY 21 (DOWN SYNDROME)

Down syndrome is caused by an extra copy of **chromosome 21 (trisomy 21)** (Fig. 2.8). Features of patients with Down syndrome include growth retardation; varying degrees of intellectual disability; craniofacial abnormalities, including upward slanting eyes, epicanthal folds (extra skin folds at the medial corners of the eyes), flat facies, and small ears; cardiac defects; and hypotonia (Fig. 2.9). These individuals also have an increased chance of developing leukemia, infections, thyroid dysfunction, and premature aging. Furthermore, an increased frequency and earlier onset of Alzheimer disease is observed among persons with Down syndrome. In 95% of cases, the syndrome is caused by trisomy 21 resulting from meiotic nondisjunction, and in 75% of these instances, nondisjunction occurs during **oocyte formation**. The incidence of Down syndrome is approximately 1 in 2,000 conceptuses for women under age 25 years. This risk increases with maternal age to 1 in 300 at age 35 years and 1 in 100 at age 40 years.

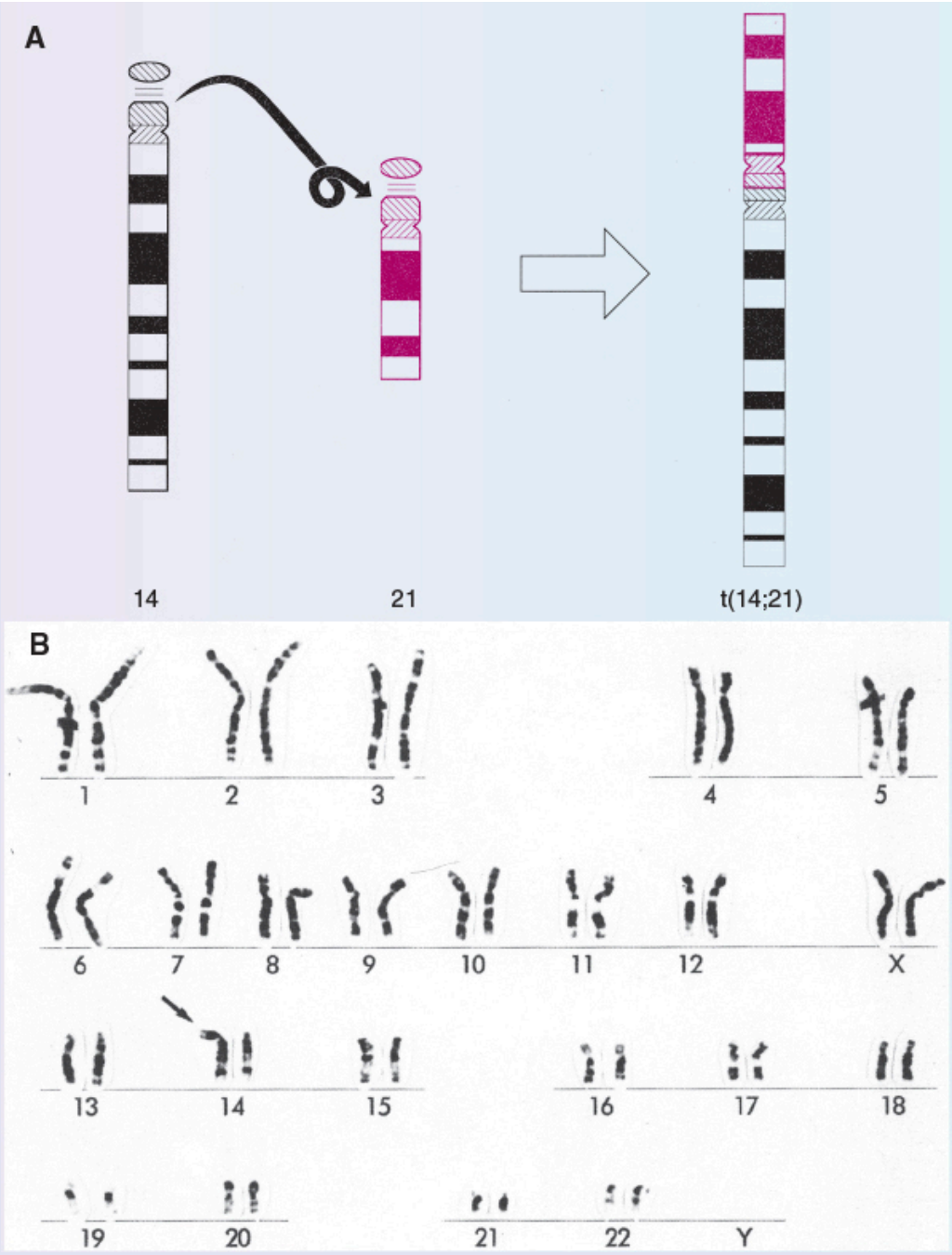


FIGURE 2.7 **A.** Translocation of the long arms of chromosomes 14 and 21 at the centromere. Loss of the short arms is not clinically significant, and these individuals are clinically normal, although they are at risk for producing offspring with unbalanced translocations. **B. Karyotype** of translocation of chromosome 21 onto 14, resulting in Down syndrome.

In approximately 4% of cases of Down syndrome, there is an unbalanced translocation between chromosome 21 and chromosomes 13, 14, 15, or 21 (Fig. 2.7). The final 1% is caused by mosaicism resulting from a trisomic conception followed by the loss of the extra chromosome in some cells during mitosis. These

individuals have **mosaicism**, with some cells having a normal chromosome number and some having trisomy. They may exhibit few or many of the characteristics of Down syndrome.

Trisomy 18

Patients with **trisomy 18** show the following features: intellectual disability, congenital heart defects, low-set ears, and flexion of fingers and hands (Fig. 2.10). In addition, patients frequently show micrognathia, renal anomalies, syndactyly, and malformations of the skeletal system. The incidence of this condition is approximately 1 in 5,000 newborns. Eighty-five percent are lost between 10 weeks of gestation and term, whereas those born alive usually die by 2 months of age. Approximately 5% live beyond 1 year.

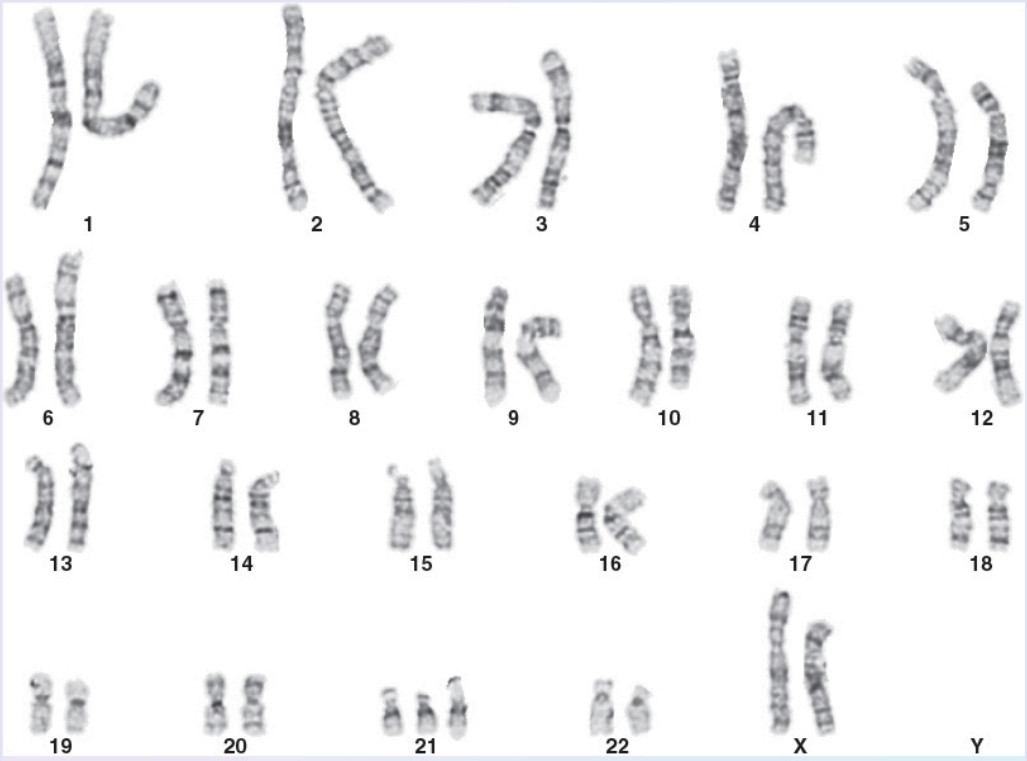


FIGURE 2.8 Karyotype of trisomy 21, Down syndrome.

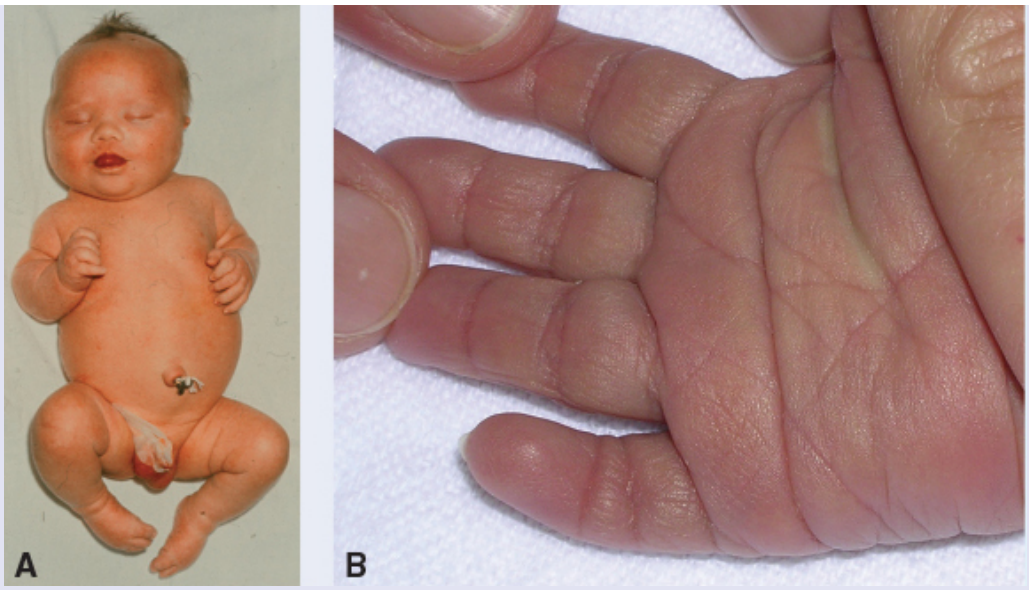


FIGURE 2.9 **A.** Child with Down syndrome. Note the flat broad face, oblique palpebral fissures, and protruding tongue. Children with Down syndrome usually have some degree of intellectual disability and many have cardiac defects. **B.** Another characteristic of these children is a broad hand with a single transverse (simian) crease.

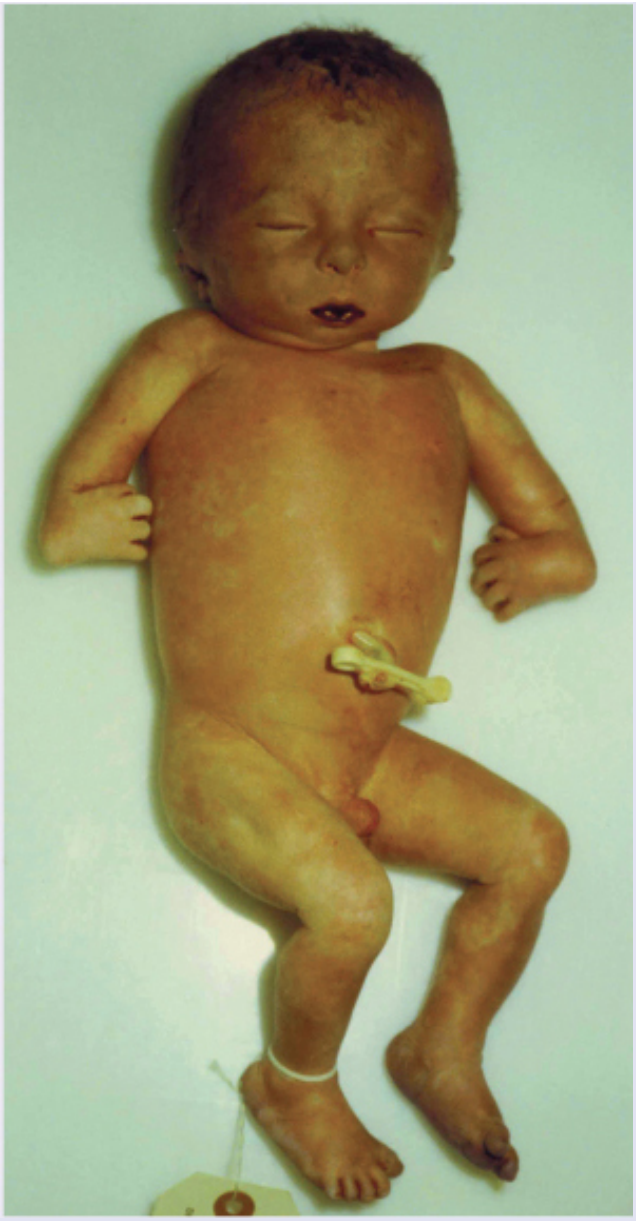


FIGURE 2.10 Child with trisomy 18. Note the low-set ears, small mouth, deficient mandible (micrognathia), flexion of the hands, and absent and/or hypoplasia of the radius and ulna.

Trisomy 13

The main abnormalities of **trisomy 13** are intellectual disability, holoprosencephaly, congenital heart defects, deafness, cleft lip and palate, and eye defects, such as microphthalmia, anophthalmia, and coloboma (Fig. 2.11). The incidence of this abnormality is approximately 1 in 20,000 live births, and more than 90% of the infants die in the first month after birth. Approximately 5% live beyond 1 year.

Klinefelter Syndrome

The clinical features of **Klinefelter syndrome**, found only in males and usually detected by amniocentesis, are sterility, testicular atrophy, hyalinization of the seminiferous tubules, and usually gynecomastia. The cells have 47 chromosomes with a sex chromosomal complement of the XXY type, and a **sex chromatin (Barr) body** is found in 80% of cases. (**Barr body**: formed by condensation of an inactivated X chromosome; a Barr body is also present in normal females because one of the X chromosomes is normally inactivated). The incidence is approximately 1 in 500 males. Nondisjunction of the XX homologues is the most common causative event. Occasionally, patients with Klinefelter syndrome have 48 chromosomes: 44 autosomes and 4 sex chromosomes (48,XXXY). Although intellectual disability is not generally part of the syndrome, the more X chromosomes there are, the more likely there will be some degree of cognitive impairment.

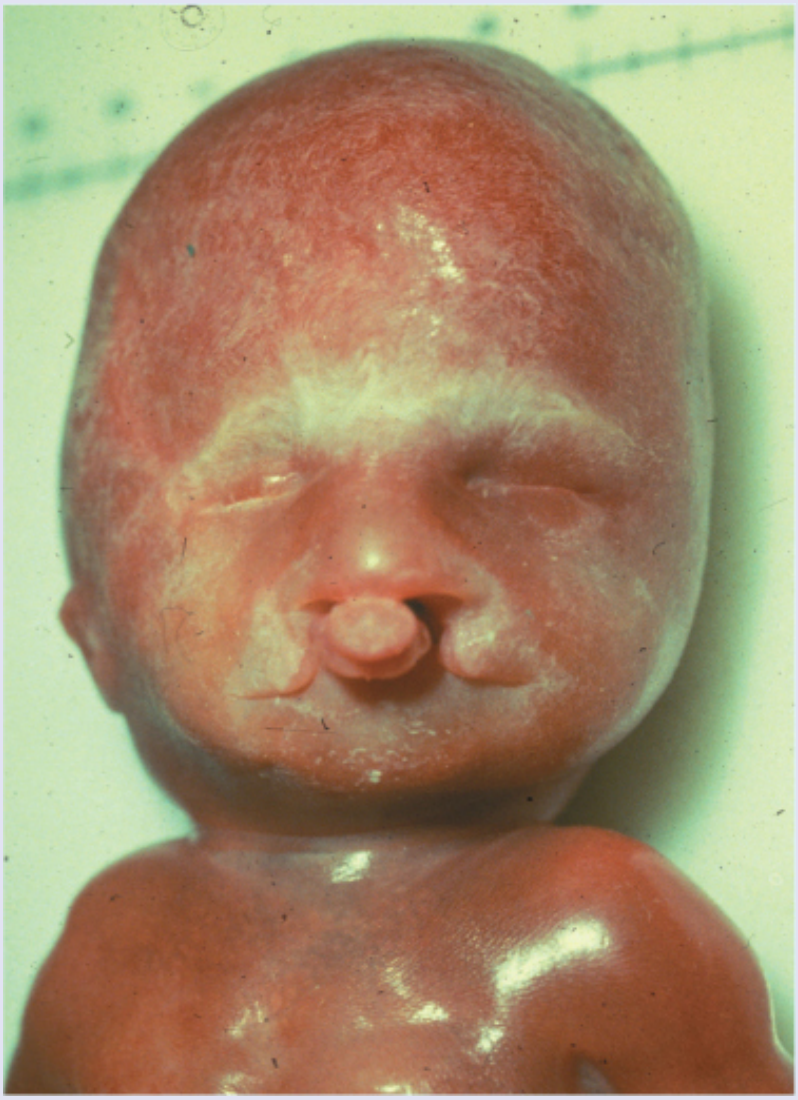


FIGURE 2.11 Child with trisomy 13. Note the bilateral cleft lip, the sloping forehead, and anophthalmia.

Turner Syndrome

Turner syndrome, with a 45,X karyotype, is the only monosomy compatible with life. Even then, 98% of all fetuses with the syndrome are spontaneously aborted. The few that survive are unmistakably female in appearance (Fig. 2.12) and are characterized by the absence of ovaries (**gonadal dysgenesis**) and short stature. Other common associated abnormalities are webbed neck, lymphedema of the extremities, skeletal deformities, and a broad chest with widely spaced nipples. Approximately 55% of affected females are monosomic for the X and chromatin body negative because of nondisjunction. In 80% of these females, nondisjunction in the **male gamete** is the cause. In the remainder of females, structural abnormalities of the X chromosome or mitotic nondisjunction resulting in mosaicism is the cause.

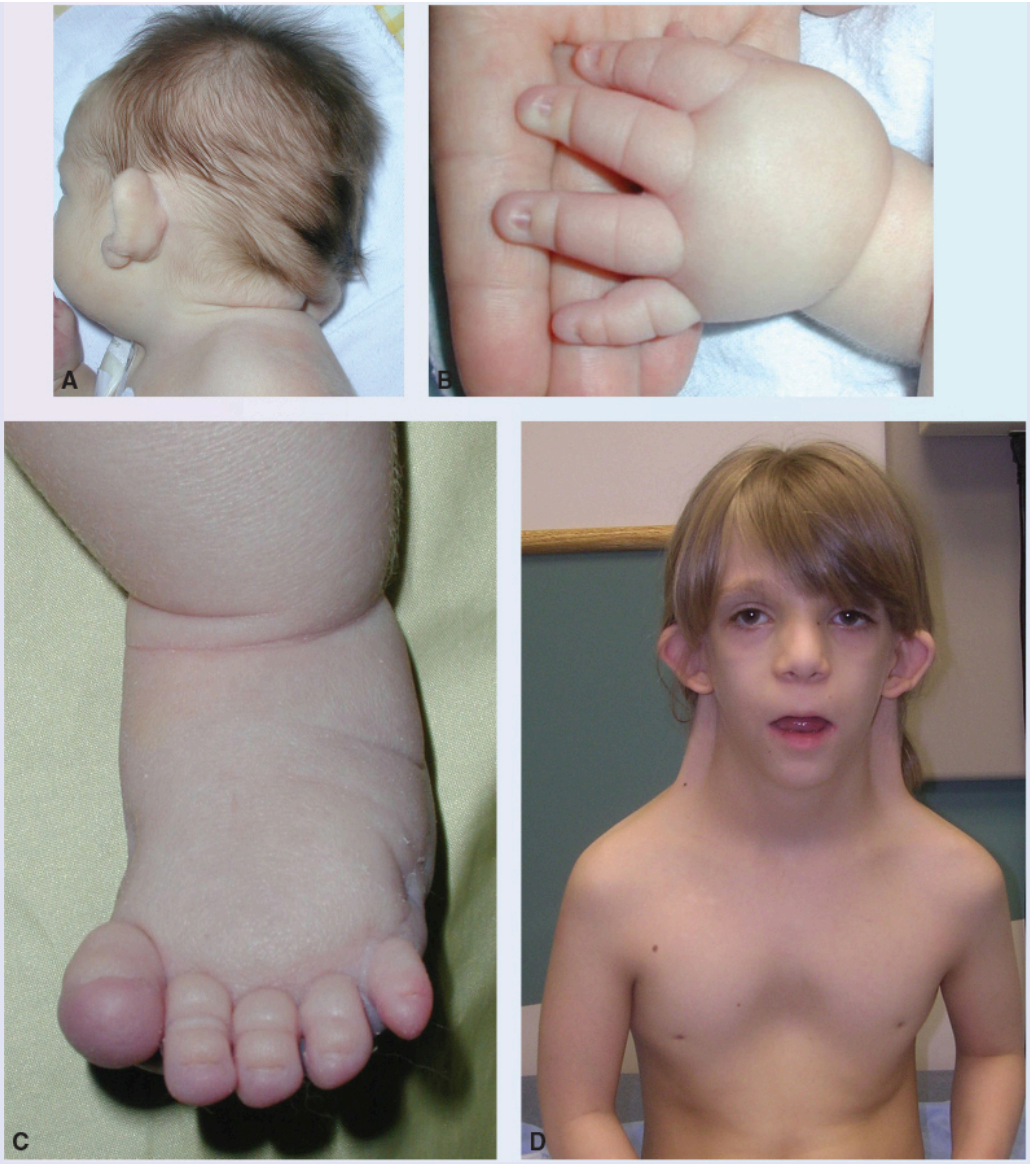


FIGURE 2.12 Patient with Turner syndrome. **A.** At birth. Note the loose skin at the posterior of the neck caused by the remains of a cystic hygroma (fluid-filled cyst), the short neck, malformed ears, and swelling in the hand **(B)** and the foot **(C)** caused by lymphedema. **D.** At 6 years of age, the webbed neck is prominent, and the nipples are widely spaced with a broad chest.

Triple X Syndrome

Patients with **triple X syndrome (47,XXX)** often go undiagnosed because of their mild physical features. However, these girls frequently have problems with speech and self-esteem. They have two sex chromatin bodies in their cells.

Structural Abnormalities

Structural chromosome abnormalities, which involve one or more chromosomes, usually result from chromosome breakage. It has been suggested that breaks are caused by environmental factors, such as viruses, radiation, and drugs, but the

evidence is inconclusive. The result of breakage depends on what happens to the broken pieces. In some cases, the broken piece of a chromosome is lost, and the infant with partial **deletion** of a chromosome is abnormal. A well-known syndrome, caused by partial deletion of the short arm of chromosome 5, is the **cri-du-chat syndrome**. Affected infants have a cat-like cry, microcephaly (small head), intellectual disability, and congenital heart disease. Many other relatively rare syndromes are known to result from a partial chromosome deletion.

Microdeletions, spanning only a few **contiguous genes**, may result in **microdeletion syndrome** or **contiguous gene syndrome**. Sites where these deletions occur, called **contiguous gene complexes**, are usually identified by **fluorescence in situ hybridization** (**FISH**; see p. 25). An example of a microdeletion occurs on the long arm of chromosome 15 (15q11–15q13). (Note: Chromosomes have a long arm, designated "q," and a short arm, designated "p," based on the position of the centromere.) When the microdeletion occurs on the maternal chromosome, it results in **Angelman syndrome**, and the children have intellectual disability, cannot speak, exhibit poor motor development, and are prone to unprovoked and prolonged periods of laughter (Fig. 2.13). If the microdeletion occurs on the paternal chromosome, **Prader–Willi syndrome** results. Affected individuals are characterized by hypotonia, obesity, intellectual disability, hypogonadism, and undescended testes (Fig. 2.14). Characteristics that are differentially expressed depending upon whether the genetic material is inherited from the mother or the father are examples of **genomic imprinting**. Other contiguous gene syndromes may be inherited from either parent, including **Miller–Dieker syndrome** (lissencephaly, developmental delay, seizures, and cardiac and facial abnormalities resulting from a deletion at 17p13) and most cases of **22q11 syndrome** (palatal defects, conotruncal heart defects, speech delay, learning disorders, and schizophrenia-like disorder resulting from a deletion in 22q11).

Fragile sites are regions of chromosomes that demonstrate a propensity to separate or break under certain cell manipulations. For example, fragile sites can be revealed by culturing lymphocytes from a patient in folate-deficient medium. Although numerous fragile sites have been defined and consist of **CGG repeats**, only those in the **FMRI** gene on the long arm of the X chromosome (Xq27) have been correlated with an altered phenotype that is called the **fragile X syndrome**. Greater than 200 repeats occur in the promoter region of the gene in affected individuals compared to 6 to 54 repeats in normal subjects. Fragile X syndrome is characterized by intellectual disability, large ears, prominent jaw, and large testes. The syndrome occurs in 1 per 5,000 individuals, and because it is an X-linked condition, males are affected almost exclusively, which may account for the preponderance of males among the cognitively impaired. Fragile X syndrome is second only to Down syndrome as a cause of intellectual disability due to genetic abnormalities.

FIGURE 2.13 Patient with Angelman syndrome resulting from a microdeletion on maternal chromosome 15. If the defect is inherited on the paternal chromosome, Prader–Willi syndrome occurs (Fig. 2.14).

Gene Mutations

Many congenital malformations in humans are inherited, and some show a clear Mendelian pattern of inheritance. Many birth defects are directly attributable to a change in the structure or function of a single gene, hence the name **single gene mutation**.

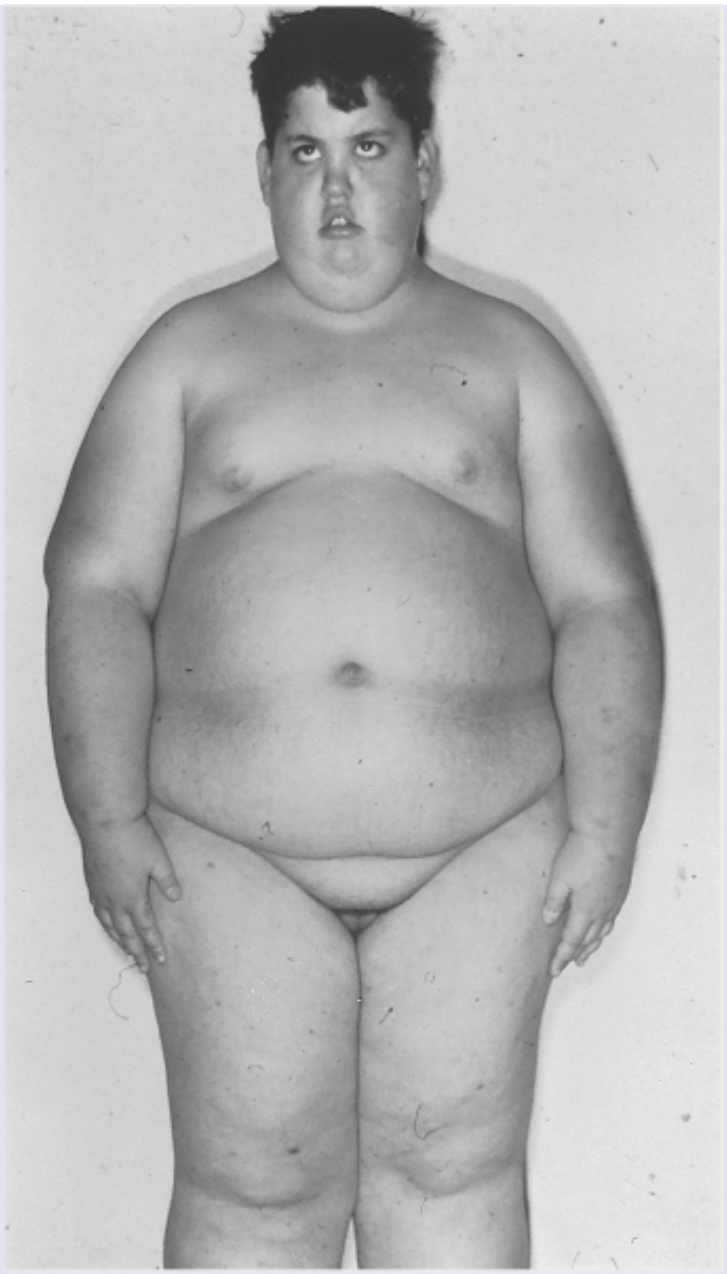


FIGURE 2.14 Patient with Prader–Willi syndrome resulting from a microdeletion on paternal chromosome 15. If the defect is inherited on the maternal chromosome, Angelman syndrome occurs (Fig. 2.13).

With the exception of the X and Y chromosomes in the male, genes exist as pairs, or **alleles**, so that there are two doses for each genetic determinant: one from the mother and one from the father. If a mutant gene produces an abnormality in a single dose, despite the presence of a normal allele, it is a **dominant mutation**. If both alleles must be abnormal (double dose) or if the mutation is X-linked (occurs on the X chromosome) in the male, it is a **recessive mutation**. Variations in the effects of mutant genes may be a result of **modifying factors**.

In some cases, mutations occur in a cell as an embryo is developing. If the mutation occurs in a somatic cell, the individual will have **mosaicism** (having more than one genetically distinct cell line) with some cells having the mutation and some not. If the mutation occurs in a germline cell (egg or sperm), the result is germline mosaicism. In this case, the parent does not express an abnormality or disease because his or her somatic cells are normal. However, the parent can transmit the defect to multiple offspring.

The application of molecular biologic techniques has increased our knowledge of genes responsible for normal development. In turn, genetic analysis of human syndromes has shown that mutations in many of these same genes are responsible for some congenital abnormalities and childhood diseases. Thus, the link between key genes in development and their role in clinical syndromes is becoming clearer.

In addition to causing congenital malformations, mutations can result in **inborn errors of metabolism**. These diseases, among which **phenylketonuria**, **homocystinuria**, and **galactosemia** are best known, may be accompanied by or cause various degrees of intellectual disability if proper diets and medical care are not instituted.

Diagnostic Techniques for Identifying Genetic Abnormalities

Cytogenetic analysis is used to assess chromosome number and integrity. The technique requires dividing cells, which usually means establishing cell cultures that are arrested in metaphase by chemical treatment. Chromosomes are **Giemsa-stained** to reveal light and dark banding patterns (G-bands; Fig. 2.8) unique for each chromosome. Each band represents 5 to 10 × 10^6 base pairs of DNA, which may include a few to several hundred genes. **High-resolution metaphase banding techniques** have been developed that demonstrate greater numbers of bands representing even smaller pieces of DNA, thereby facilitating diagnosis of small deletions.

Molecular techniques, such as **fluorescence in situ hybridization (FISH)**, use specific DNA probes to identify ploidy for a few selected chromosomes and for detecting microdeletions. Fluorescent probes are hybridized to chromosomes or genetic loci using cells on a slide, and the results are visualized with a fluorescence microscope (Fig. 2.15).

Microarrays use spots of specific DNA sequences (probes) attached to a solid surface, usually glass or silicon (Affymetrix chips). These probes may be a short sequence from a gene or other DNA element that are used to hybridize a cDNA or cRNA sample (the target sample). Hybridization of probe–target sequences is detected and quantified using fluorescence or other reporter techniques. Results can detect single nucleotide polymorphisms, mutations, and changes in expression levels. Some companies now offer such techniques commercially for anyone who wants their genome tested or sequenced.

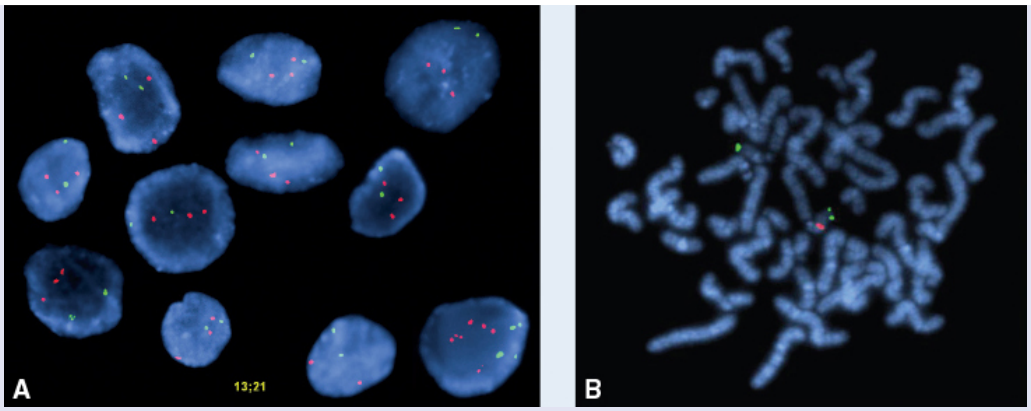


FIGURE 2.15 A. FISH, using a probe for chromosome 21 (*red dots*). Note that there are three *red dots* in each cell, indicating trisomy 21 (Down syndrome). The *green dots* represent a control probe for chromosome 13. Two cells are superimposed on the lower right, giving the impression of the presence of multiple probes. **B.** FISH analysis of 22q11 deletion syndrome. The green signals identify chromosome 22; the red signal represents FISH probe N25, which is in the q11 region. It is present on only one of the pairs of chromosome 22, indicating the other has the 22q11 deletion.

Exome sequencing represents a new approach to finding **mutations** and **polymorphisms** (single nucleotide polymorphisms [SNPs] in a DNA sequence) responsible for birth defects and diseases. With this technique, only the coding regions (exons) in the genome are sequenced. Since together, these coding regions make up the **exome** and represent only 1% to 2% of the entire human genome, sequencing them is more practical than trying to sequence the entire genome. Because most genetic variants lie within the coding regions for proteins, this technique is an efficient way to discover these differences. This technique is also superior to older approaches that relied on linkage studies followed by positional cloning (searching for candidate genes in specific regions of chromosomes) because these techniques required large numbers of affected individuals within a family and could not be used to study affected individuals from different families. In contrast, exome sequencing can find a causative mutation in a single affected individual if the exomes from both parents can also be sequenced. Even sequencing affected individuals from different families regardless of kinship can be successful. It must be remembered, however, that exome sequencing can only identify variants in the coding regions of genes that alter proteins. Other genetic causes of birth defects that lie outside the coding region have to be identified by **whole genome sequencing.** As costs continue to decrease, sequencing the entire genome will become much more feasible.

MORPHOLOGIC CHANGES DURING MATURATION OF THE GAMETES

Oogenesis

Oogenesis is the process whereby oogonia differentiate into mature oocytes.

Maturation of Oocytes Begins Before Birth

Once **PGCs** have arrived in the gonad of a genetic female, they differentiate into **oogonia** (Fig. 2.16A,B). These cells undergo a number of mitotic divisions, and by the end of the third month, they are arranged in clusters surrounded by a layer of flat epithelial cells (Figs. 2.17 and 2.18). Whereas all of the oogonia in one cluster are probably derived from a single cell, the flat epithelial cells, known as **follicular cells**, originate from surface epithelium covering the ovary.

The majority of oogonia continue to divide by mitosis, but some of them arrest their cell division in prophase of meiosis I and form **primary oocytes** (Figs. 2.16C and 2.17A). During the next few months, oogonia increase rapidly in number, and by the fifth month of prenatal development, the total number of germ cells in the ovary reaches its maximum, estimated at 7 million. At this time, cell death begins, and many oogonia as well as primary oocytes become **atretic** (degenerate). By the seventh month, the majority of oogonia have become atretic except for a few near the surface. All surviving primary oocytes have entered prophase of meiosis I, and most of them are individually surrounded by a layer of flat follicular epithelial cells (Fig. 2.17B). A primary oocyte, together with its surrounding flat epithelial cells, is known as a **primordial follicle** (Fig. 2.18A).

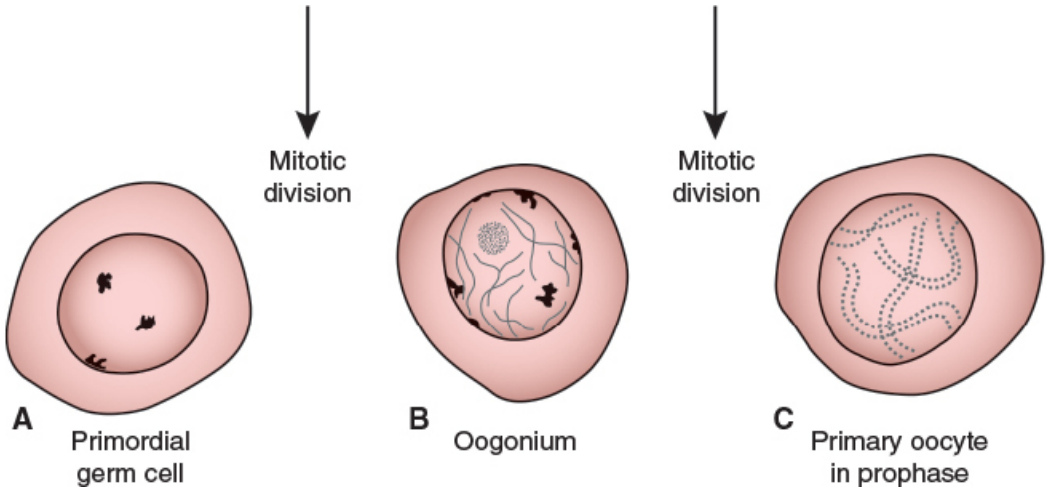


FIGURE 2.16 Differentiation of PGCs into oogonia begins shortly after their arrival in the ovary. By the third month of development, some oogonia give rise to primary oocytes that enter prophase of the first meiotic division. This prophase may last 40 or more years and finishes only when the cell begins its final maturation. During this period, it carries 46 double-structured chromosomes.

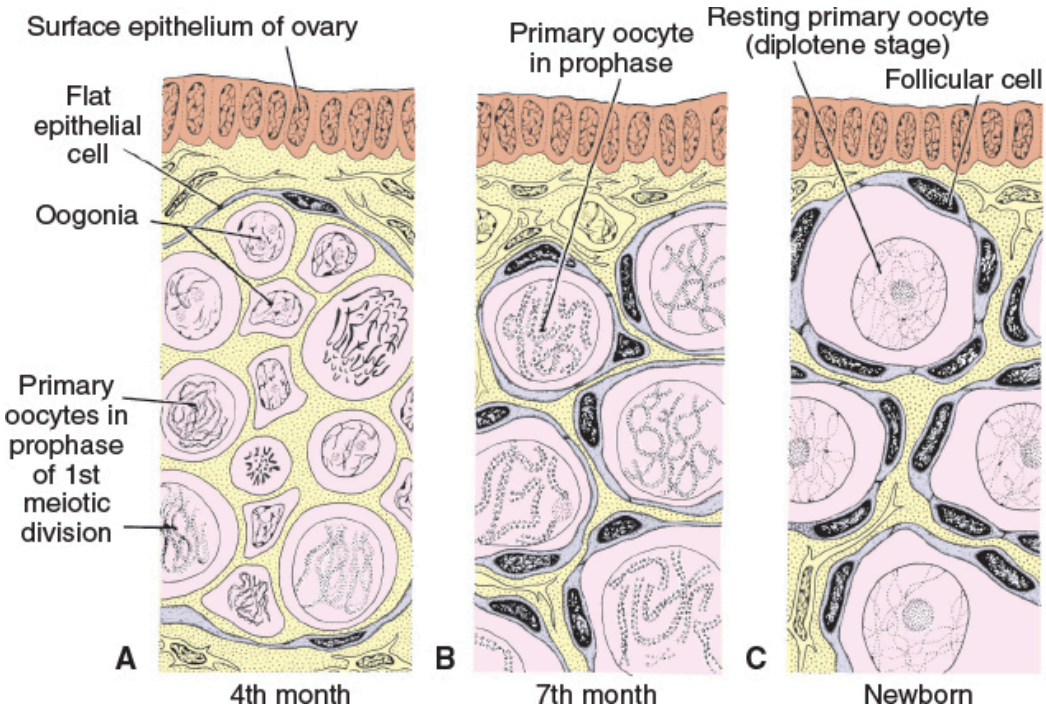


FIGURE 2.17 Segment of the ovary at different stages of development. **A.** Oogonia are grouped in clusters in the cortical part of the ovary. Some show mitosis; others have differentiated into primary oocytes and entered prophase of the first meiotic division. **B.** Almost all oogonia are transformed into primary oocytes in prophase of the first meiotic division. **C.** There are no oogonia. Each primary oocyte is surrounded by a single layer of follicular cells, forming the primordial follicle. Oocytes have entered the diplotene stage of prophase, in which they remain until just before ovulation. Only then do they enter metaphase of the first meiotic division.