Part 1

General Embryology

Introduction to Molecular Regulation and Signaling

1

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**,

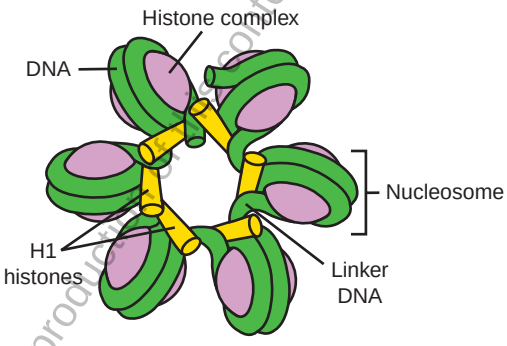

FIGURE 1.1 Drawing showing nucleosomes that form the basic unit of chromatin. Each nucleosome consists of an octamer of histone proteins and approximately 140 base pairs of DNA. Nucleosomes are joined into clusters by linker DNA and other histone proteins.

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**.

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

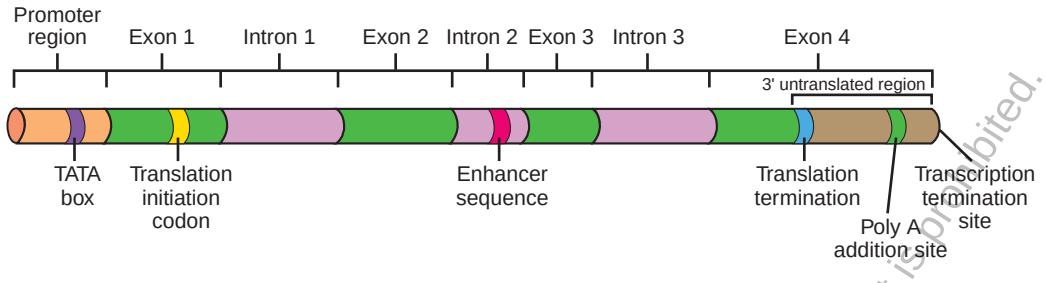

FIGURE 1.2 Drawing of a "typical" gene showing the promoter region containing the TATA box; exons that contain DNA sequences that are translated into proteins; introns; the transcription initiation site; the translation initiation site that designates the code for the first amino acid in a protein; and 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.

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.

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

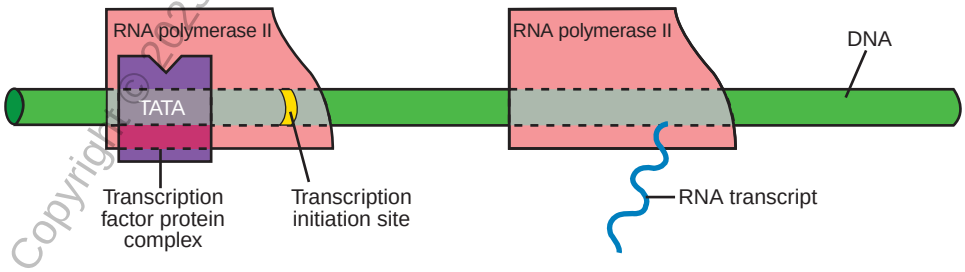

FIGURE 1.3 Drawing showing binding of RNA polymerase II to the TATA box site of the promoter region of a gene. This binding requires a complex of proteins plus an additional protein called a *transcription factor*. Transcription factors have their own specific DNA-binding domain and function to regulate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

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.

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

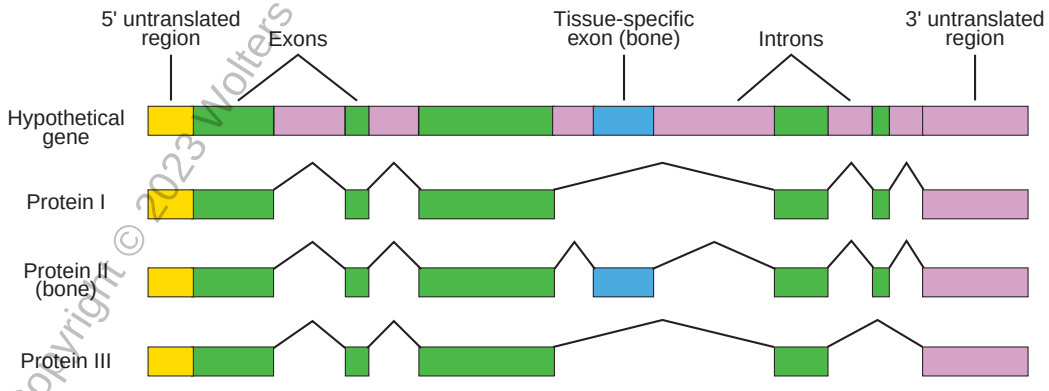

FIGURE 1.4 Drawing of a hypothetical gene illustrating the process of alternative splicing to form different proteins from the same gene. Spliceosomes recognize specific sites on the initial transcript of nRNA from a gene. Based on these sites, different introns are "spliced out" to create more than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

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.

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*).

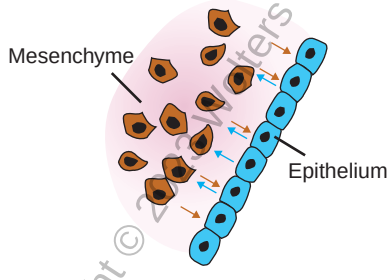

FIGURE 1.5 Drawing illustrating an epithelial–mesenchymal interaction. Following an initial signal from one tissue, a second tissue is induced to differentiate into a specific structure. The first tissue constitutes the inducer, and the second is the responder. Once the induction process is initiated, 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).

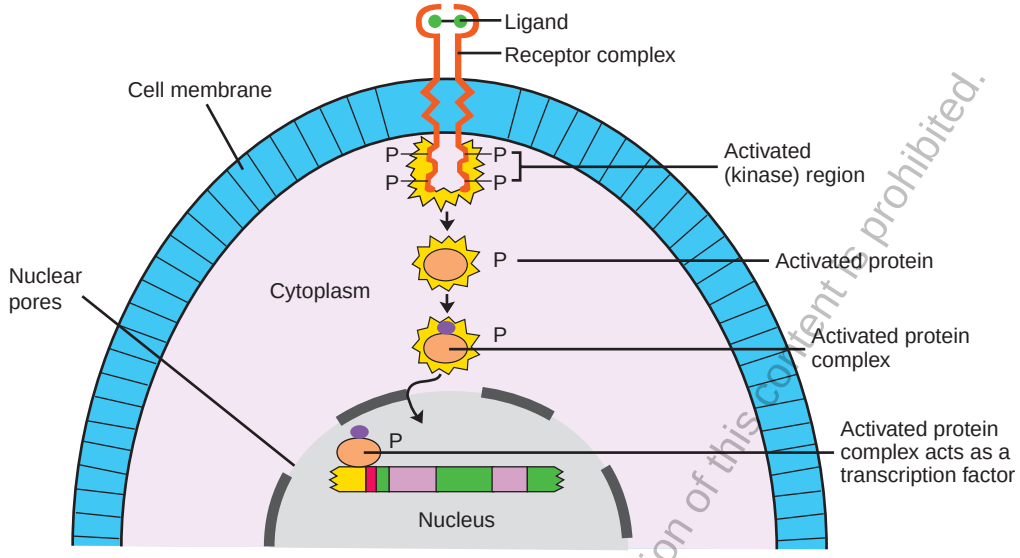

FIGURE 1.6 Drawing of a typical signal transduction pathway involving a ligand and its receptor. Activation of the receptor is conferred by binding to the ligand. Typically, the activation is enzymatic involving a tyrosine kinase, although other enzymes may be employed. Ultimately, kinase activity results 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

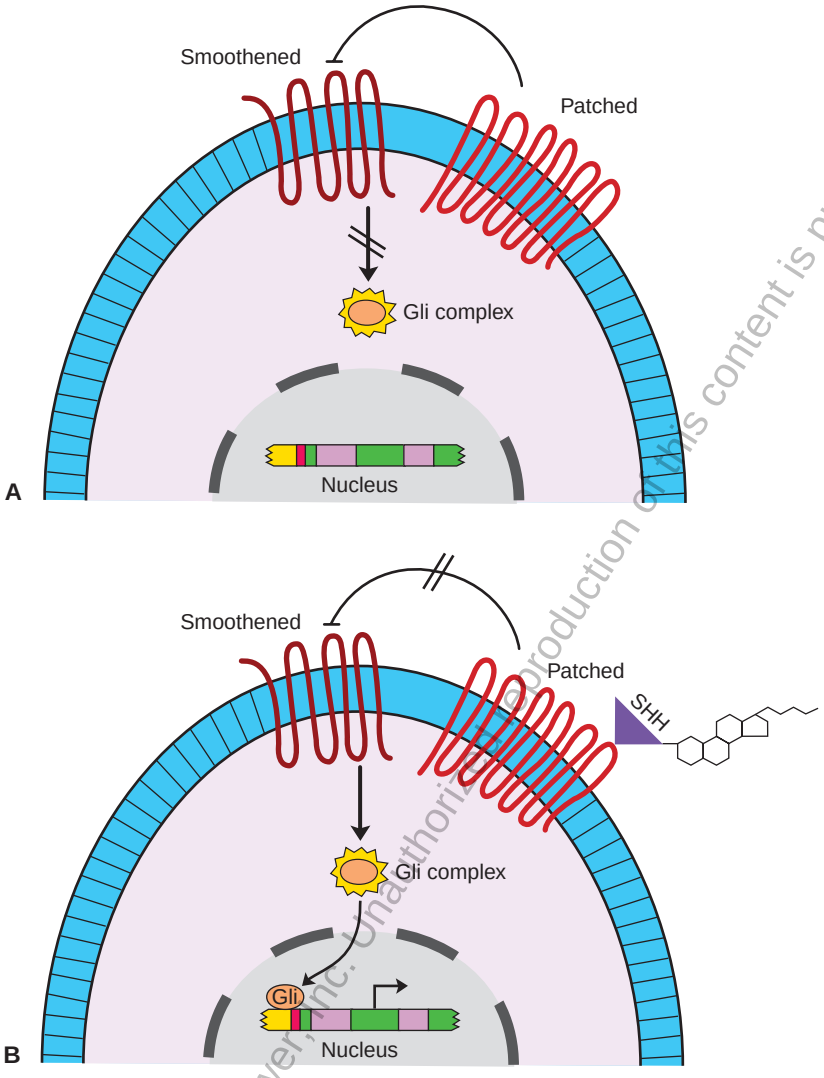

FIGURE 1.7 Drawings illustrating the sonic hedgehog (SHH) signaling pathway. **A.** Drawing of a cell showing Patched inhibition of Smoothened that blocks activation of the GLI proteins that normally transduce the SHH signal. **B.** Drawing showing SHH binding to its receptor Patched, that removes Patched 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.

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).

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

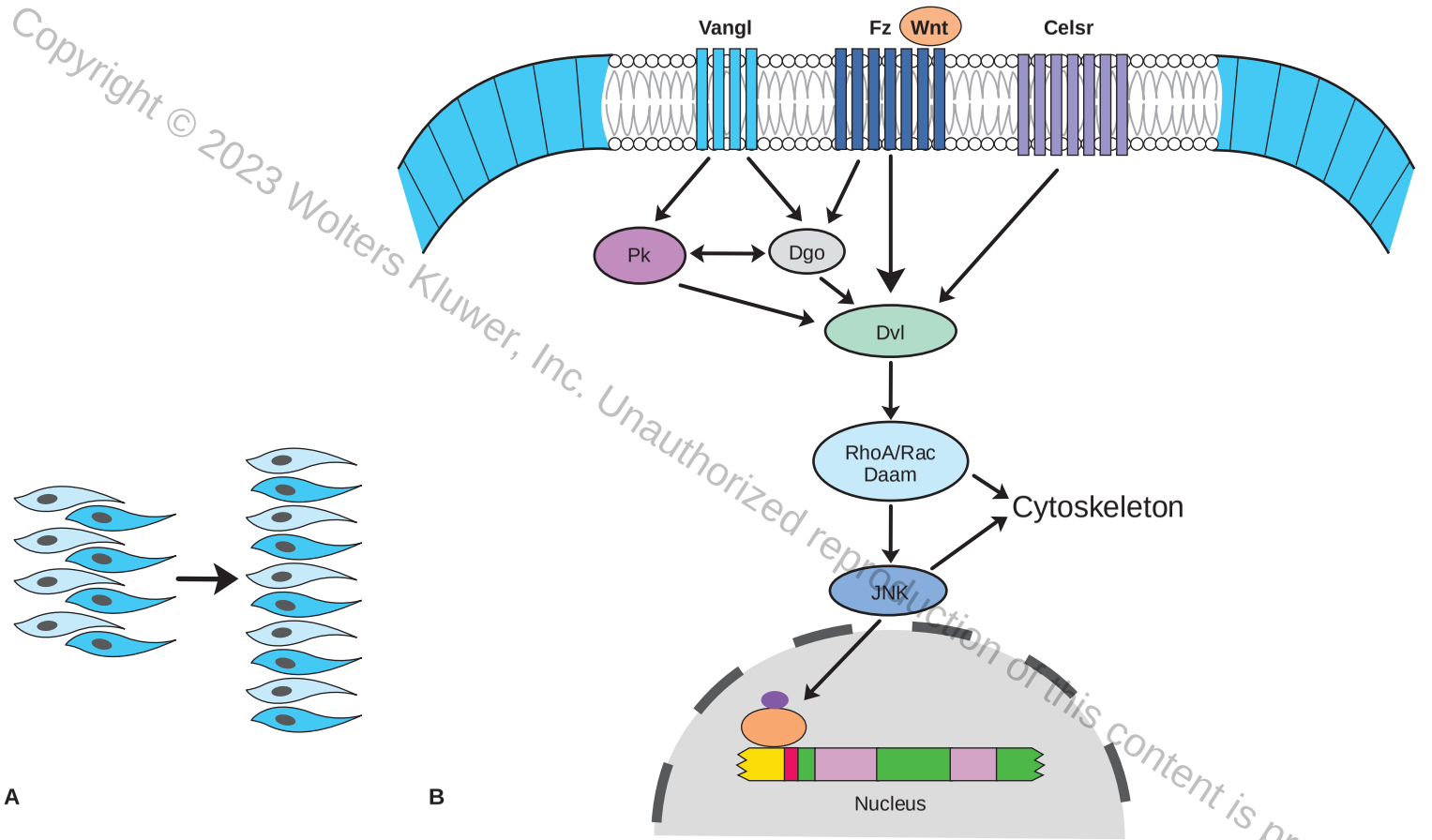

FIGURE 1.8 **A.** Drawing illustrating the process of convergent extension whereby cells intercalate with their neighbors to increase the long axis of a tissue, such as occurs during lengthening of the neural tube during neurulation. Convergent extension is dependent on the PCP pathway (the reorganization 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.

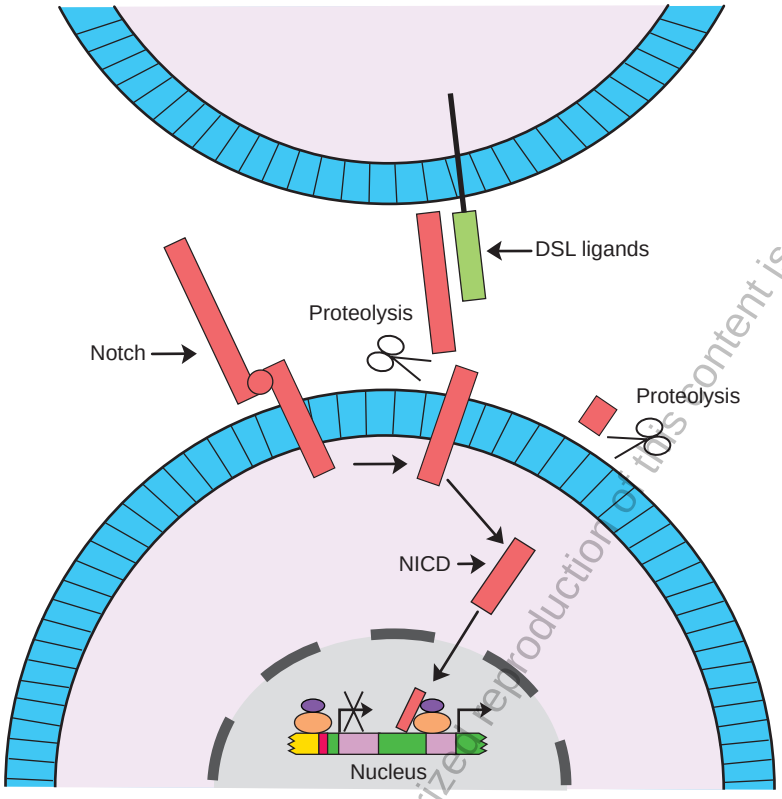

FIGURE 1.9 Drawing illustrating signaling via the *Notch* pathway. Notch receptors located on one cell bind a ligand from the *DSL* family (Jagged or Serrate) that are located on an adjacent cell (juxtacrine signaling), and this receptor–ligand interaction activates a proteolytic enzyme that cleaves 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.

of tetralogy of Fallot (a cardiac outflow tract defect).

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?