INTEGRATED HUMAN EMBRYOLOGY

Colección Salud

Saldarriaga Gil, Wilmar
Integrated human embryology /Wilmar Saldarriaga Gil, Carolina Isaza de Lourido, Julián Ramírez Cheyne. -- Cali : Programa Editorial Universidad del Valle, 2020.
286 páginas ; 28 cm. -- (Colección Salud)
1. Embriología humana - 2. Desarrollo embrionario - 3. Espermatogénesis - 4. Desarrollo fetal - 5. Diagnóstico prenatal - 6. Anomalías congénitas

612.64 cd 22 ed.
S162

Universidad del Valle - Biblioteca Mario Carvajal

Universidad del Valle
Programa Editorial
Title: Integrated human embryology
Autors: Wilmar Saldarriaga Gil, Carolina Isaza de Lourido, Julián Ramírez Cheyne
Traductors: Wilmar Saldarriaga Gil, Claudia Valencia Peña
ISBN-PDF: 978-958-5144-82-8
Collection: Salud
First edition in Spanish 2015
First edition in English

Principal: Edgar Varela Barrios
Vice Chancellor for Research: Jaime R. Cantera Kintz
Head of the Programa Editorial: Omar J. Díaz Saldaña

Layout and illustrations: Hugo H. Ordóñez Nievas, Isabella Manjarrés Melo
Collaborators: Laura Alejandra Ávila Vidal, Manuel Alejandro Vásquez Salguero

---

Cali, Colombia, october 2020

INTEGRATED HUMAN EMBRYOLOGY

Wilmar Saldarriaga Gil
• Carolina Isaza de Lourido •
Julián Ramírez Cheyne

Colección Salud

PREFACE

When I received professor Saldarriaga request to elaborate the preface of the book called *Integrated human embryology,* which he and Dr. Carolina Isaza de Lourido wrote, I thought I would find a dense content text, focused on the description of morphological changes that gametes undergo so that fertilization can occur, and the changes that happen posteriorly until the embryonic period ends. I couldn't imagine the amount and depth of the information given in this text about the genetic basis; molecular expression of genes that generate proteins and enzymes which lead normal cell differentiation processes, regulate sperm and zygote formation; fertilization as properly said; development of the embryo, and the consequences of an altered protein expression, which are the cause of many birth defects.

The book can be divided into four big sections, the first one provides the reader anatomical, physiological, endocrine and molecular foundations about differentiation and maturation of male and female gametes, that end up with the formation of an haploid cell in preparation for fertilization. Describes in detail fertilization process that originates a new individual, with a unique genetic material obtained from the merge of the genetic material invested by the father and the mother, followed up by a series of molecular mechanisms that result in the development of a new individual. Also describes in detail molecular biology processes that originate, lead and execute transcription and genetic expression, that establish intracellular enzyme and protein production and cell differentiation processes of multiple visceral structures developing in the embryo. All of these chapters are written with great detail and linked in a very clear and coherent way.

The second section describes the process that takes place during the first and up to the eighth week of human embryo development. These chapters not only approach morphological changes that the zygote undergoes in order to become and embryo, as classically described in embryology books, it also engages in a complete way description of molecular structures that support embryoblast and trophoblast division processes, endometrium preparation for implantation and its invasion; immuno-

logical aspects that avoid conceptus rejection, differentiation of the layers that originate all the embryo tissues: ectoderm, mesoderm, and endoderm; the establishment of body axes, gastrulation, embryo folds, neurulation, somite and endoderm development. Also tackles interesting topics for the obstetrics clinician, such as the causes of early abortion, action of some contraceptives, ectopic pregnancy, early pregnancy diagnosis based on placental hormone production and morphological findings using ultrasound, during normal pregnancy or in early birth defect cases.

Also it begins the description of birth defects as the outcome of failures during normal genetic transcription processes (genetic or chromosomal anomalies), changes in the role or structure of biochemical messengers, cell division and migration alterations, or as a consequence of a external noxa (toxic, infectious or abnormal morphological development of the amnios, placental or fetal tissue).

The third section approaches aspects of fetal and new born function, as well as the placenta. Similar to the past sections, this section not only describes changes in organ systems already developed, how the role of each organ starts, or how they vary during the pregnancy and changes at birth, but also contributes valuable information about the factors that support these changes, even if they are genetic or endocrine. This section provides the foundation of fetal growth defects of early and late onset. Also, the authors contribute with interesting topics for the obstetrics field and hand a morphological correlation through fetal and placenta ultrasound images.

Finally, the authors engage birth defects topic through a classification, diagnosis and counselor optic. We are in front of a text that matches in a simple and clear way a great amount of useful information for medical students, postgraduate basic sciences students, like geneticists and the ones dedicated to molecular biology, or obstetrics and pediatrics residents, and as a consult tool for obstetrics, perinatology and pediatrics physicians.

I encourage you to dive into embryonary and fetal development study, its foundations in genetics, molecules and biochemistry, not just the structure, but also their roles and the consequences of a bad development or improper function; advising superficially about the proper approach to individuals with birth defects and their families.

I congratulate the authors not only for risking it by thinking outside the box when engaging classic concepts of the general embryology, but for proving a useful text for different audiences.

Hernando Gaitán D.
Profesor titular
Departamento de Obstetricia y Ginecología
Universidad Nacional de Colombia
Editor *Revista Colombiana de Obstetricia y Ginecología*

ACKNOWLEDGMENTS

To all the medical and dentistry students that encourage the writing of this book by requesting bibliography for their studies before and after lectures; to the patients and parents of the cases that allowed image publishing; to the professors and colleagues from the Universidad del Valle; to the Programa Editorial of the Universidad el Valle.

To my wife and children: Claudia Valencia, Manuela y Juan Diego Saldarriaga, who besides inspiring the book contributed taking *in utero* images.

AUTHORS AND COLLABORATORS

Wilmar Saldarriaga Gil MD., MSc., Ph.D.
Gynecologist-obstetrician. Biomedical Sciences.
Medical Genetics.
Associate Professor
Research Vice-dean
Health Faculty, Universidad del Valle

Claudia Valencia Peña MD., ESP.
Ophthalmologist. Glaucomatologist.
Ph.D. Student
Biomedical Sciences
Assistant professor
Universidad del Valle

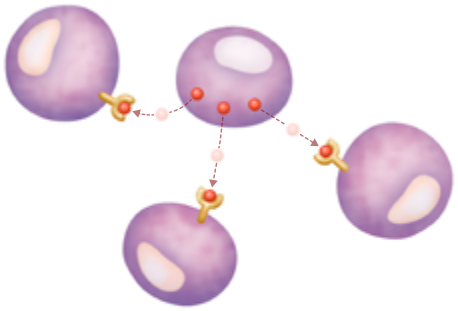
Felipe Ruiz Botero MD., MSc.
Biomedical Sciences, Medical Genetics.
Assistant profesor Universidad del Valle
Researcher at Universidad ICESI

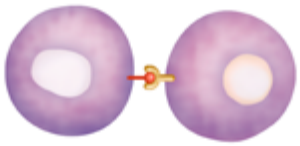
Laura Camila Molina Barrera MD.
Physician and surgeon
Universidad del Valle

Julián Ramírez Cheyne MD., MSc
Biomedical Sciences, Medical Genetics.
Assistant professor
Universidad del Valle

Carolina Isaza de Lourido MD., MSc.
Morphology.
Retired Associate Professor
Universidad del Valle
Medical director
DIME Neurovascular clinic

CONTENT





CHAPTER 1

CELLULAR REGULATION AND SIGNALING

Felipe Ruiz Botero • Wilmar Saldarriaga Gil • Claudia Valencia

INTRODUCTION

The constant development of methodologies in molecular biology has allowed advances of great importance on the chemical composition and cellular processes, particularly in physicochemical properties, genome and genes functions and the regulation of their expression in terms of RNA and protein production, allowing a better understanding of normal and pathological processes in human development[1-2]. Hence, possible interventions are proposed in order to correct biological processes and prevent genetic diseases or congenital anomalies.

The knowledge generated from the development of molecular techniques has allowed to investigate the genetic regulation of human development; which, along with the sequencing of the human genome, has led to expand the knowledge of embryology, going from anatomical and biochemical development to molecular induction.

The Human Genome Project allowed to identify an estimated of 20.000 to 25.000 genes in human DNA. However, the number of proteins that are encoded by these genes is greater, leading us to a new concept, that a single nucleotide sequence (gene) can code for different proteins through various mechanisms[1].

Gene expression is regulated in several levels[1]:

- In the transcription process, during which genetic information contained in the DNA is transcribed to messenger RNA (mRNA) molecules.
- Through the selective processing of transcribed mRNA during which the produced messenger reaches the cytoplasm in order to be translated, producing different proteins according to cellular specialization by means of a process called alternative splicing.
- mRNA can be selectively translated according to the function and cellular needs.
- Proteins made from mRNA can be differentially modified
- When synthesized proteins are differentially chemically modified in order to fulfill their specific function.

It is important to keep in mind that there are initiatives like the ENCODE project (ENCyclopedia of DNA Elements) which aims to identify all the functional elements that are encoded in the human genome. This project considers as gene every element of DNA which has been attributed with, in addition to its biological protein-coding function, many other functions like gene expression regulation, maintenance of chromosome structure and the mediation of DNA replication dynamics.[3]

GENE TRANSCRIPTION

Genes are the functional unit of inheritance; every gene is a nucleotide sequence inside the genome that gives rise to a RNA transcript, genes can or cannot be translated into a protein. The basic gene behaviour was defined by Mendel more than a century ago, this behaviour was summarized in the laws of segregation, dominance and independent assortment. A gene can exist in alternative sequences, these variations are called alleles[4].

In diploid organisms, with two set of chromosomes, each parent passes a set to their offspring; this principle also rules at gene level, where one copy of a gene corresponds to a paternal allele and the other to a maternal allele. Mentioned inheritance pattern led to the discovery that chromosomes are formed by genes[4] (see figure 1.1).

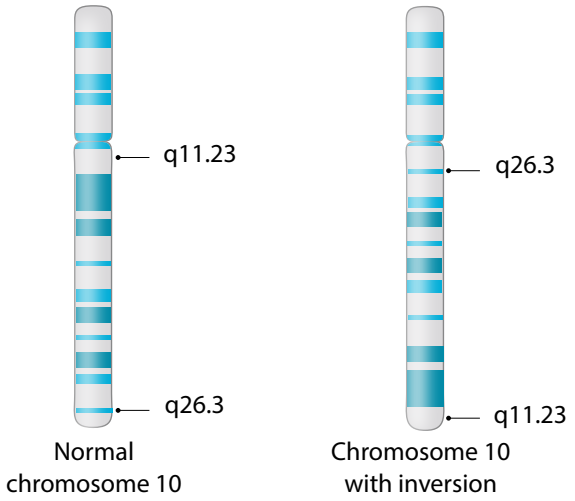


Fig. 1.1. **Chromosomal inversion.** Normal human chromosome 10 at the left and a chromosome 10 with q arm paracentric inversion at the right.

Each chromosome is a linear arrangement of a sort of genes, each of them is part of a DNA sequence and has a particular position (locus).

DNA and genes are organized and packaged in a quaternary structure called chromatin, which is located inside the nucleus of eukaryotic cells and is constituted by DNA and proteins, most of them are histones, the nucleosome is its basic structural unit[1, 4]. The nucleosome is a histone octamer (2H2a,

2H2b, 2H3, 2H4) around which, 200 bp of DNA coil.

Nucleosomes are linked by naked DNA (linker DNA), on top of which, the H1 histone is located and keeps the DNA coiled.

Chromatin has a compact organization in which most of the DNA sequences are structurally inaccessible and functionally inactive. This suggests that DNA can't be completely organized this way, it needs a functional and hierarchical arrangement[1, 5].

This concept includes two physical states of chromatin: the less condensed or *euchromatin* which codes for proteins and the most condensed, called *heterochromatin* (Figures 1.2 and 1.3); in the latter, two types can be distinguished: the more condensed or constitutive heterochromatin, which is not expressed and occupies regions in chromosomes such as the centromere; the other type of heterochromatin is termed *facultative heterochromatin* which can undergo regulation processes to become euchromatin and therefore be transcribed[1, 5-6].

In the euchromatin state (less condensed DNA), chromatin structure seems like a pearl necklace, the pearls represent nucleosomes. DNA needs to be uncoiled from nucleosomes in order to be transcribed[1] (see figures 1.2 and 1.3). In humans, the genome is constituted by 46 chromosomes located in somatic cells and 23 chromosomes in gametes[6].

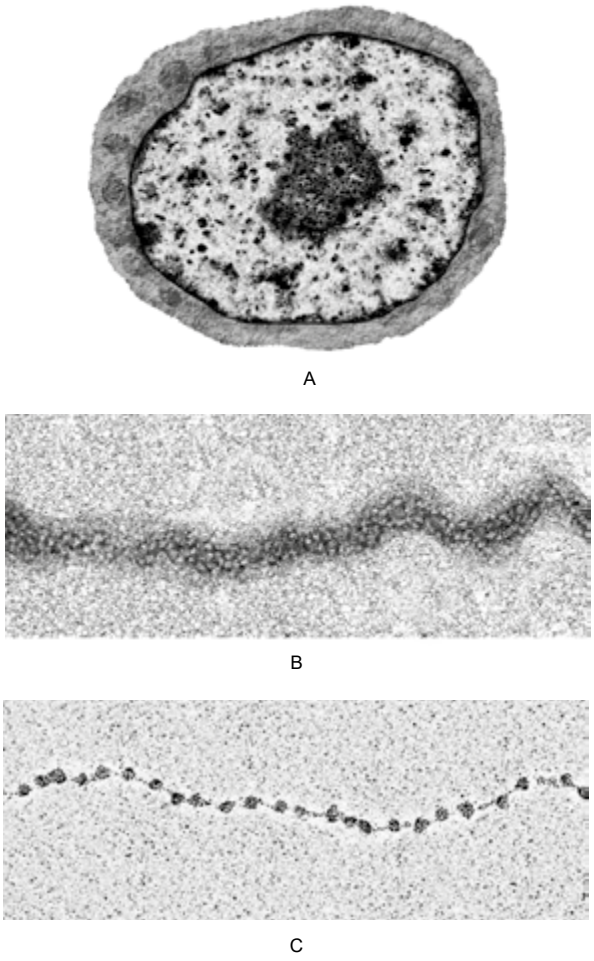


Fig. 1.2. **DNA condensation phases.** A. Micrograph where the different DNA condensation phases in the nucleus are shown: heterochromatin and euchromatin. B-C. Micrographs that show heterochromatin and euchromatin respectively using a close up view.

GENES

Genes are the functional and storage units of genetic information of organisms in the biological world, they are present in cellular DNA and the majority of them are transcribed into RNA molecules. [7-9] (messenger RNAs, transfer RNAs, ribosomal RNAs, etc.).

In DNA molecules genes are nucleotide sequences that are transcribed into messenger RNA (mRNA) through the activity of the RNA polymerase enzyme. Initially, the DNA nucleosome complex unwinds and proteins called transcription factors recognize an specific domain or initiation site on the DNA called promoter region, which may contain a sequence known as TATA box, and eases the

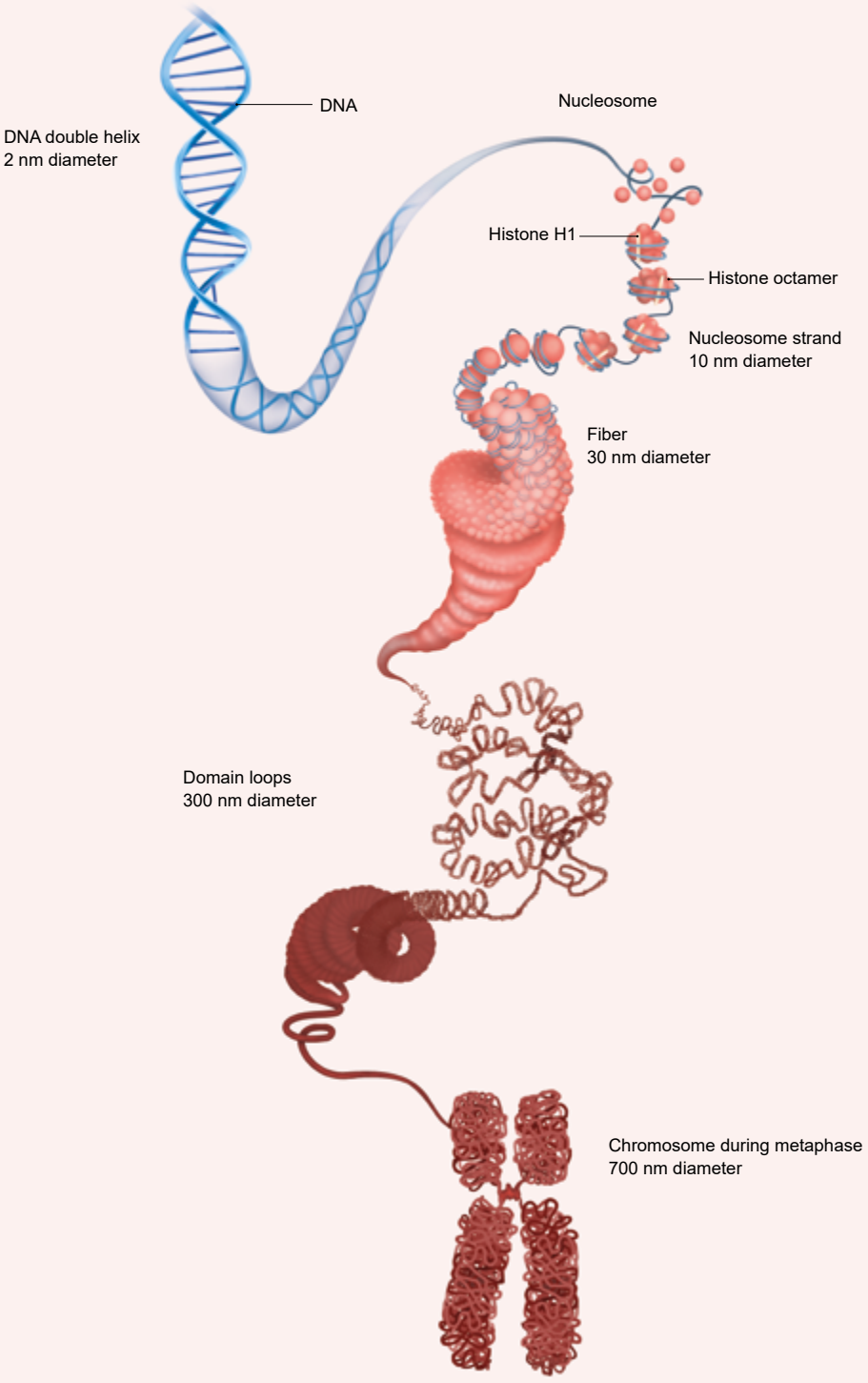


Fig. 1.3. **DNA folding.** DNA double helix chain is folded around histone proteins creating nucleosomes. The sequence of nucleosomes is called nucleosomal filament, this filament folds over itself adopting a spiral shape, where in each lap there are six nucleosomes, making up a solenoid. DNA of cells during interphase is usually found in a euchromatin state, as a nucleosomal filament. Whereas when is found in a heterochromatin state it is packed as a solenoid. Mitosis phase has superior folding levels, the solenoid organizes as a structural loop domains that contain among 20 000 and 70 000 of base pairs. These loops can fold over themselves and generate the condensation level of a mitotic chromosome, visible through optical microscopy.

arrive of RNA polymerase enzyme to DNA which is responsible for the transcription of the corresponding gene (RNA synthesis) and it also synthetizes a complementary RNA copy of the gene. RNA synthesis is done from 5' → 3', through a 3' → 5' read of the DNA strand[10-11].

The biological process of translating mature mRNA nucleotide sequence into an encoded protein is done in the 5′ → 3' sense. In this process, each triplet of nucleotides (codon) corresponds to an amino acid of the protein according to the genetic code. Mature mRNA will have a protector (which is the 7-Methylguanosine), an start codon, the nucleotide sequence that will be translated, a stop codon and a 3' untranslated region, this region includes a sequence that assist in mRNA stabilization (Poly A addition site), eases its exit from the nucleus and allows it to be translated into a protein[1, 12-13].

By convention, 5' and 3' gene regions are specified in relation to the RNA transcribed from the gene. Also, DNA is transcribed from 5' → 3' and the promoter region is located upstream the transcription start site. The need for precision probably explains why DNA replication occurs in the 5' → 3' direction[1]. If DNA polymerase added deoxyribonucleotide triphosphates in 3' → 5' direction, the growing 5' chain would provide the activation triphosphate needed for covalent bonds formation thus, polymerization errors couldn't be simply corrected with a hydrolysis process[1].

The promoter region, where the binding to RNA polymerase occurs, usually contains the TATA sequence (TATA box). For the binding of the polymerase to this site, the action of additional proteins known as transcription factors is needed, these are adaptor molecules that detect regulatory sequences in DNA and orchestrate the assembly of protein complexes that control gene expression[1, 14].

Transcription factors also have a specific DNA-binding domain and a transactivating domain which activates or inhibits the transcription of the gene whose promoter (or enhancer) it has recognized. In combination with other proteins, transcription factors activate gene expression by uncoiling the DNA nucleosome complex, by releasing the polymerase (so that it can transcribe the DNA template) and by preventing the formation of new nucleosomes[1, 12, 15].

Enhancers are other important DNA sequences, they are DNA regulating regions that activate the use of promoter sequences in order to control the efficiency and the rate of gene transcription. Enhancers can reside in any site along the DNA strand and don't need to be located near to the promoter sequence.

Like promoters, *enhancers* bind to transcription factors (through the transactivating domain of the transcription factor) and are used to regulate the time of expression of a gene and its specific cell localization. For instance, different enhancers of a gene can be used to direct that the same gene is expressed in different tissues[1, 8]. An example of the above mentioned feature is the PAX6 transcription factor, which participates in the development of the neural tube, the pancreas and the eye. This transcription factor recognizes different enhancers, each of them regulates the expression of the gene in the appropriate tissue.

Enhancers act by altering chromatin in order to expose the promoter or by easing the binding of the RNA polymerase. Sometimes, these enhancers can inhibit transcription and are known as silencers. This event allows a transcription factor to activate a gene while silencing another by binding to different

enhancers. Thus, transcription factors have a specific domain on a DNA region in addition to a transactivating domain that binds to a promoter or enhancer and subsequently activates or inhibits a gene by regulating these elements[1].

A gene might have fragments called exons which are separated by DNA sequences known as introns, these sequences will not be translated and have to be eliminated; throughout this process, resulting exons fuse (splicing process) and make up a single mature messenger RNA molecule that will go out towards the cytoplasm to be translated into a protein[1, 5]. Also, it has been shown that depending on the cell type, introns removal differs among cells and therefore the protein product of the messenger RNA translation also variates[12, 16-17].

TRANSCRIPTION FACTORS

Genes that code for proteins reside in the nucleotide sequences that are present in the DNA strand and will later be transcribed inside the cell nucleus into messenger RNA (mRNA) molecules, as it was previously described. The process needs molecular families that act as transcription factors. Some of them may be general factors which are present in most of the cells of an organism, and others are specific factors that fulfil functions in certain cell types or in certain development stages, usually, they're essential for the initiation of gene expression patterns.

Transcription factors are subdivided in homeodomain proteins and homeobox sequence based upon their DNA interaction mechanisms and structure[18].

In humans, 39 *Hox* genes have been identified in four *clusters* in chromosomes 2, 7, 12 and 17. These proteins have a homeodomain with a high grade of conservation which is constituted of 60 amino acids of the helix-loop-helix type. The homeodomain gene is encoded by 180 nucleotides which are called homeobox[18].

Hox genes

Hox genes play a major role in craniocaudal segmentation. These genes are activated in a strict sequence in the 3'-> 5' direction and following their positions on the chromosomes. Although *Hox* genes were initially described to have functions along the main body axis, like in the hindbrain development and the appearance of the rhombomere, their sequential expression has also been observed in developing organs or regions, among which bowel, limbs, blood cells, internal and external genitalia are included[18].

The main function of the *Hox* genes is to set up diverse structures along the main body axis; secondarily, these genes can later participate in the formation of specific non-axial structures. Mutations of these genes lead to morphological changes in the segmental structures, in which a specific gene is usually expressed[18].

Pax genes

Pax genes are a family composed of nine recognized genes which are involved in several aspects of development in mammals. These genes are responsible of many notable functions in the nervous system, sense organs and also participate in cellular differentiation processes that entail epithelial-mesenchymal transitions; they have an important role in several organs, for example, the pancreas and the eye[18].

Dlx genes

Dlx genes have a high phylogenetic conservation grade. These genes play important

roles in processes of establishing the corporal patterns in early stages of embryonic development and in the jaws and inner ear morphogenesis. In mammals, these genes act in pairs and with a narrow association with Hox genes[18].

Msx genes

This family has two representatives in humans. *Msx* proteins play important roles in embryonic development, especially in epithelial-mesenchymal interactions of the limbs and the face. Msx proteins are general inhibitors of cellular differentiation in prenatal development and mantain the proliferative capacity of tissues in the postnatal period[18].

T-box gene family

T-Box genes (*TBX*) are a gene family with over 100 members, these genes play important roles in development: induction of the mesoderm layer, specification of the mesodermal germ layer and specification of developing limbs (leg or arm); also, *T-Box* genes participate in heart development. These genes contain a conserved region (T-box) which encodes 180 to 200 amino acids that are bound to a specific nucleotide sequence of DNA[18].

Other gene families that contain homeobox

The *POU* genes family. The name of this family derives from the acronym of the first identified genes (Pit-1, Oct-1, Oct-2, Unt-86). Genes from this family have a homeobox, which is a region that encodes 75 amino acids[18].

Lim proteins participate in different phases of the whole-body formation. It is a large family; some of these proteins are located in the cytoplasm and others bind to DNA[18].

HELIX-LOOP-HELIX TRANSCRIPTION FACTORS

Basic Helix-Loop-Helix proteins

These are proteins in which there's a region where two α-helices are separated by a loop of amino acids. This region plus another basic region of the protein allow the regulatory protein to bind to a specific DNA sequence. The Helix-Loop-helix region participates in homodimerization or heterodimerization processes[18.]

Forkhead gene family (*FOX* genes)

These genes make up a transcription factors family of over 100 members. *FOX* genes are expressed throughout the development of many organs. These genes have microscopic domains inside the developing organ and can act jointly to guide the morphogenesis of a structure[18].

The deregulation of genes from the *FOX* gene family is responsible for birth defects, diabetes mellitus and carcinogenesis. The knowledge of processes such as expression forms, gene alterations and epigenetic changes of genes that interact with Fox family transcription factors, will probably allow the development of preventive and therapeutic tools[19].

Zinc finger transcription factors

This family is made of proteins in which cysteine (Cys) and histidine (His) units are linked by zinc ions. This feature allows the polypeptide chain to fold into finger-like structures that can interact with specific regions of the DNA helix[18].

Fox genes

The members of this large family have in common an HMG (*high mobility group*)

domain in their proteins which is infrequent in transcription factors. Sox proteins were discovered in 1990 when it was shown that *SRY* gene was the male-determining factor in sex differentiation. These proteins act together with other transcription factors, modifying the expression of their target genes. Sox proteins are expressed in most of structures throughout development, specifically, they have an important role in sex differentiation[18].

WT1

The Wilms tumor suppressor gene (*WT1*) is a crucial gene for initial morphological development of the kidney and for its development in adults. Likewise, this gene has a relevant role in the gonads formation[18].

METHYLATION

DNA can be covalently modified; this allows the regulation of gene expression. In cells of organisms that belong to the subphylum Vertebrata, cytosine methylation provides a powerful mechanism by which gene expressions patterns are passed to the cells of the offspring (see Figure 1.4) [20].

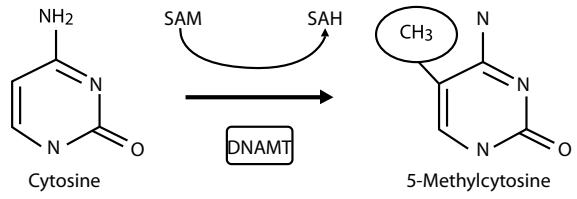


Fig. 1.4 DNA methylation occurs at the 5 position of the cytosine ring; SAM: S-Adenosyl methionine, methyl group donator; SAH: S-Adenosyl-L-methionine; DNAMT: DNA methyltransferase.

DNA methylation in vertebrates is almost exclusive to cytosine nucleotides in the CpG sequence. This simple mechanism allows the DNA methylation pattern to be directly inherited by DNA daughter strands[20]. DNA methylation has many uses in vertebrate cells but, maybe the most important use is to work in concert with other gene expression mechanisms in order to set an efficient gene silencing form that can be passed to the cells of the offspring[20].

Methylation silences DNA by inhibiting the binding of transcription factors or by altering the DNA interaction with histones octamers in the nulceosome; methylation is one of the main epigenetic mechanisms. Methyl groups are transferred in some of the cytosines (C) of CpG sequences. For example, in case that total and permanent deactivation of a gene is required, DNA is methylated without altering its sequence, there's a modification in the histones of the nucleosomes by adding methyl, acetyl, phosphate groups, etc. Since the epigenetic mechanism interfere in gene silencing, it is of utmost importance in developmental biology. Also, proteins, which are gene products, can be methylated by enzymes, with a regulation of their function.

Methylation of cytosines in the promoter region of the gene suppresses their transcription; thus, some genes are silenced by this mechanism. For instance, one of the X chromosomes in each cell of female individuals is inactivated (X chromosome inactivation) by this mechanism, methylation. Similarly, genes in different cell types are silenced through methylation, for example, genes that are responsible for blood proteins in muscle cells; in these cells, genes that are in charge of producing muscle proteins won't

be methylated. In this way, each cell can maintain their differential characteristics[1, 20].

DNA methylation is also responsible for genomic imprinting by which a gene inherited from the father or the mother is expressed while the other is silenced. Approximately 40 to 60 human genes are imprinted and their methylation patterns are set during spermatogenesis and oogenesis. Methylation silences DNA by inhibiting the binding of transcription factors or by altering the binding to histones, causing nucleosome stabilization and a tightly coiled DNA that can't be transcribed[1, 20].

Some examples of syndromes that can be generated due to an alteration in methylation are Beckwith Wiedemann syndrome, Prader Willi, Angelman, Rett, among others; such alteration also makes part of the molecular bases of cancer. Alterations in methylation are also known as imprinting alterations or epigenetic diseases.

OTHER REGULATORS

The initial transcript of a gene is called nuclear RNA (nRNA) or sometimes premessenger RNA. nRNA is longer than mRNA because it contains introns which are removed (spliced out) when nRNA moves from the nucleus to the cytoplasm, in fact, this splicing process provides a mechanism for cells to produce different proteins from a single gene. For instance, by removing different introns, exons are "spliced out"[2] in different patterns, this process is known as *alternative splicing*[1] (see Figure 1.5).

Alternative splicing is done by spliceosomes, which are complexes between small nuclear RNA (snRNA) and proteins that recognize specific splice sites in 5' or 3' nRNA ends. Proteins that are derived from the same gene are called splicing isoforms (splice variants or alternative splice forms) and, these allow that the same gene can be used to produce

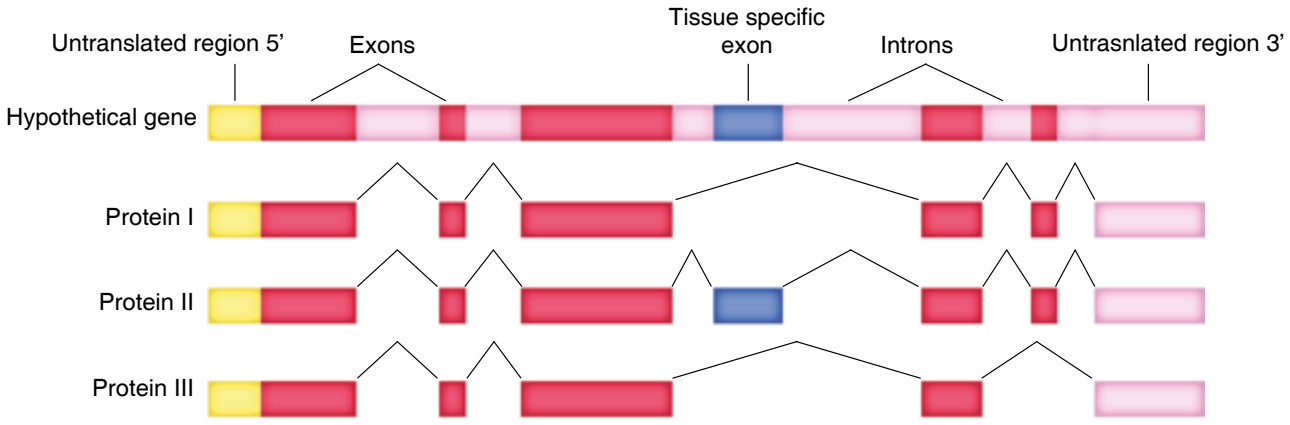


Fig 1.5. **Alternative splicing.** Diagram explaining the process where three different proteins are produced from a single gene.

Source: adapted from Sadler T, Langman J. Langman medical embriology. 13.th ed. Baltimore: Lippincott Williams & Wilkins; 2016.

specific proteins for that cell type, for example, *WT1* gene isoforms have different roles in gonadal vs kidney development[1].

Once the protein is synthesized,there may be post-translational modifications that can affect its function. For instance, some proteins have to be cleaved to become active or they might have to get phosphorylated. Others need to be combined with other proteins, be released of storage sites or be a specific target of cellular regions. Despite of the existence of only 25.000 genes, the number of proteins that have been found is probably around five to eight times the number of [1].

MOLECULAR INDUCTION OF ORGAN FORMATION

Organs are formed by the interaction between tissues and cells. Often, a group of cells or tissues makes other group of cells or tissues change their fate, this process is called induction. In each of those interactions, a cell type or tissue is the inducer, which produces a signal, and the other one is the responder of the signal. The capacity of responding to mentioned signal is called competence, and competence needs the activation of the tissue that responds to a competence factor[1].

Many of the inductive interactions occur between epithelial cells and mesenchymal cells and are known as epithelial-mesenchymal interactions. Epithelial cells join in tubes or sheets while mesenchymal cells are fibroblastic in appearance and dispersed in an extracellular matrix. Epithelial-mesenchymal interaction examples: gut endoderm and the surrounding mesenchyme to produce organs derived from the gut that include the liver (*SHH*, *HOX*), pancreas (*SHH*, PDX1) [21]; mesenchyme of limbs with the corresponding ectoderm that covers it (epithelium) to produce growth and differentiation of the limbs (*TBX4*, *TBX5*, *FGF10*), and the ureteric bud endoderm and the mesenchyme of the metanephric blastema in order to produce nephrons in the kidney (*WT1*) [1, 22].

Inductive interactions can also occur between two epithelial tissues, like the lens induction by the epithelium of the optic cup. Although, an initial signal goes from the inducer tissue to the responder tissue, the responder tissue can respond with a second molecule which leads the inducer tissue to differentiate. Thus, the dialogue between the two tissues or the two cell types is essential for differentiation to continue[1].

CELL SIGNALING

The cell-cell signaling is essential for induction and for the dialogue between inducing and responding cells. This communication is established by paracrine interactions, either by proteins synthesized and excreted by a cell which diffuse over short distances to interact with other cells or by juxtracrine interactions that don't include diffusible proteins. Diffusible proteins are responsible of paracrine signaling, they are called paracrine or growth factors and differentiation factors (GDFs) [1].

Paracrine signaling

Transcription factors act by transduction signaling pathways, either by directly activating a pathway or blocking the activity of a pathway inhibitor (inhibitor of an inhibitor, like in the case of hedgehog signaling). Signal transduction pathways include a signaling molecule (the ligand) and a receptor. The receptor is a membrane bound protein that has an extracellular domain (region

where the binding to the ligand occurs), a transmembrane domain and finally, a cytoplasmic domain[1] (see Figure 1.6).

When the ligand binds to the receptor, it induces a conformational change in the receptor, activating its cytoplasmic domain. Often, the result of this activation is conferring enzymatic activity to the receptor, and frequently, the given activity to the receptor is to act as a kinase that is able to phosphorylate other proteins using ATP as a substrate; subsequently, phosphorylation activates these proteins in order to modify additional proteins, therefore a protein interaction cascade is established which, finally, activates a transcription factor that activates or inhibits gene expression[1].

Pathways are numerous and complex, in some cases a protein that inhibits another is used and as a result a third protein is activated, e.g. the hedgehog signaling pathway[1].

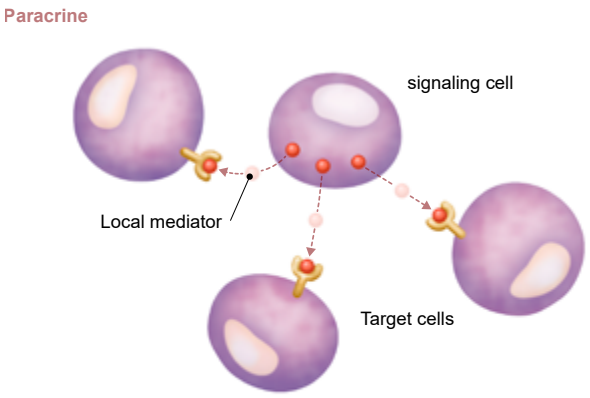


Fig. 1.6. Paracrine signaling.

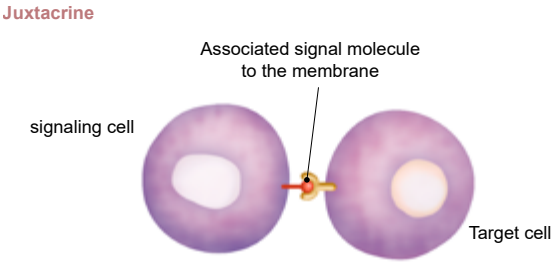


Fig 1.7. Juxtacrine signaling.

Juxtacrine signaling

Juxtacrine signaling is also mediated through signals from the signal transduction pathway, but in this case there's no need for diffusible factors. Thus, there are three pathways for juxtacrine signaling to occur[1] (see Figure 1.7).

A protein on the surface of a cell interacts with a receptor on an adjacent cell in a process that is analogous to paracrine signaling. The Notch pathway is an example of this type of signaling. The protein of the Notch receptor spreads through the cell membrane and binds to cells that have Delta, Serrate or Jagged proteins in their cell membranes. The binding of one of these proteins to Notch gives rise to conformational changes in the Notch protein, a part of its cytoplasmic domain is separated. The cleaved portion then binds to a transcription factor to activate gene expression. Notch signaling has special importance in neuron differentiation, blood vessels specification and segmentation of somites[1].

Cell secreted ligands on the extracellular matrix interact with receptors of adjacent cells. The extracellular matrix is the molecular environment in which cells reside. This environment contains large molecules that are secreted by cells, these molecules include collagen, proteoglycans (chondroitin sulfate, hyaluronic acid, etc.) and glycoproteins such as fibronectin and laminin[1].