Gene Action: Developmental Genetics

13

Stefan Mundlos

Abstract Developmental genetics studies the mechanisms how genes initiate and control the process by which a single cell can give rise to a mature organism. This includes mechanisms of early patterning, as well as later events that result in the formation and maturation of organ systems. Developmentally active genes exert their effects through many pathways and mechanisms including diffusing morphogens, cell migration, proliferation, and border formation. Transient structures such as the somites, the branchial arches and the apical ectodermal ridge serve as scaffold and signaling centers during embryogenesis. Gene defects frequently result in abnormal development with specific phenotypes that reflect the gene's essential functions during embryogenesis. In many instances this results in a combination of malformations that are characteristic for a specific syndrome.

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13.1 Genetics of Embryonal Development

The study of embryonal development focuses on the process by which a single cell can give rise to a mature organism. This involves the early steps of development that set the pattern for the overall bauplan of the body and the development of individual organs studied in model systems, such as the insect eye, the vertebrate limb, or the nervous system. The study of developmental biology, however, goes beyond the study of embryos. It includes the regeneration of lost organs, such as the newt limb, or the lizard tail, and the control of post-embryonic growth, a process that includes metamorphosis and aging. Development is less an adaptation and reaction to certain stimuli and more a programmed set of events in which the transient stages are more

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important than the permanent ones. The information for this process is contained within the genome. The genome holds the code that tells the embryo to develop and controls differentiation of cells, thus directing the entire process. But this is not a one-way process, since the embryo, on the other hand, is able to control its genome, mediating between genotype and phenotype, between the inherited genes, its environment, and the adult organism. Since gene regulation is obviously the key event in the organization of this process, many mechanisms have been proposed and experimentally verified. But how this intricate control system is able to induce and maintain differences between cells, enabling them to differentiate, remains largely unclear. The answer will probably not come from considering merely DNA and its interactions with RNA and proteins. The feedback between the cells, their genome, and the environment will have to be considered. The organism, the regulative pathway including a network of cooperating partners, and the interacting environment have to be studied as a whole for true understanding of this process to be possible. The detection of a mutation in a patient with a heart defect links the function of this gene with heart development, but how this gene contributes to a critical process during development has to be investigated in detail using functional analysis. Mutations in humans can lead to new insights into the factors that contribute to certain developmental processes, but only the study of the entire regulatory network will yield a thorough understanding that will ultimately enable us to comprehend complex human traits and their interaction with environmental stimuli.

Because human experimentation is subject to obvious limitations, appropriate model systems are needed to study the molecular basis of developmental processes. Over the past century developmental biologists have established a wide variety of model systems that have greatly contributed to our understanding of the basic mechanisms of animal and human development. These include the fruit fly *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, the sea urchin *Lytechinus variegatus*, the zebra fish *Danio rerio*, the frog *Xenopus laevis*, the chick, and the mouse, to mention the most important ones. *Drosophila* was the first animal model to be studied in great detail, because it is genetically amenable and therefore suitable for large-scale mutation screening, genetic analysis, and manipulation. Many of the mechanisms underpinning early embryonic development were established in this species and, surprisingly at the time, were found to be conserved throughout the animal kingdom. The sea urchin has been most instrumental in the study of early development, in particular gastrulation, whereas *C. elegans* is a more recent model system appreciated for its stereotyped developmental program, an almost invariant cell lineage, and easy manipulation. *Xenopus* and the chick have been used as vertebrate model organ systems because both species produce robust embryos that develop outside of the mother, allowing easy manipulation. Both systems, however, have the disadvantage that they cannot be genetically manipulated. In this sense the mouse is the preferred organism, because of its suitability for genetic manipulation (in particular gene targeting) and its closeness to humans. The zebra fish combines many of the above-mentioned advantages owing to its accessibility and genetic amenability.

13.1.1 Basic Mechanisms of Development

The problem of how the embryo determines a pattern is one of the most central questions in biology. How is the cell fate orchestrated in a three-dimensional space by a set of instructions, the genes, that are the same in every cell? This question has captivated biologists and scientists from many other disciplines who have infused the field with their viewpoints. Clearly, the embryo uses a variety of ways to determine its own gestalt and function. At the molecular level several different processes are incorporated that affect the behavior of cells. Cell *proliferation* leads to an increase in cell number and thus expansion and growth of the embryo. New structures can be generated by differentially increasing the rate of proliferation. *Growth* may also be accomplished by the synthesis of macromolecules or an increase in cell size. Programmed cell death or *apoptosis* is an important mechanism to remove transient structures. For example, our fingers and toes are created by the death of interdigital cells in the hand and foot plates. Cell *migration* is the movement of an individual cells or groups of cells with respect to other cells in the embryo, as observed, for example, in neural crest and germ cells that undergo extensive migration and consequently populate parts of the embryo that are very distant from their original locations. During further development, *differentiation* takes place, a process by which cells become structurally and functionally specialized.

Cells become organized in tissues by sticking together, a process called condensation. This can take place through the expression of complementary adhesion molecules on their surfaces, and/or they may form associations with their extracellular matrix. Adhesion molecules function in the same way as a receptor-ligand interaction, except that in this case the adjacent cells carry either the receptor or the ligand. By this mechanism borders can be induced and maintained, ensuring that the cells on either side of the border develop according to a different scheme. The delta/notch pathway represents such a system, where one cell expresses notch whereas the other carries the delta receptor on its surface, each inducing its own set of gene expression. Posttranslational modification of proteins is an important way to modify their interaction with other proteins [13]. For example, fringe, a known modifier of notch signaling, has been shown to function as a fucose-specific *N*-acetylglucosaminyltransferase, demonstrating that notch signaling can be regulated by protein modification. More recently, microRNAs, small RNA molecules that inhibit the translation of specific mRNAs, were shown to be involved in gene regulation in development. All of these mechanisms are instrumental in inducing differentiation, but how a body plan and thus a three-dimensional structure is established cannot be explained by these mechanisms alone. One emerging concept that is likely to be central to the problem is the presence of so-called morphogens, signaling molecules that determine cell fate.

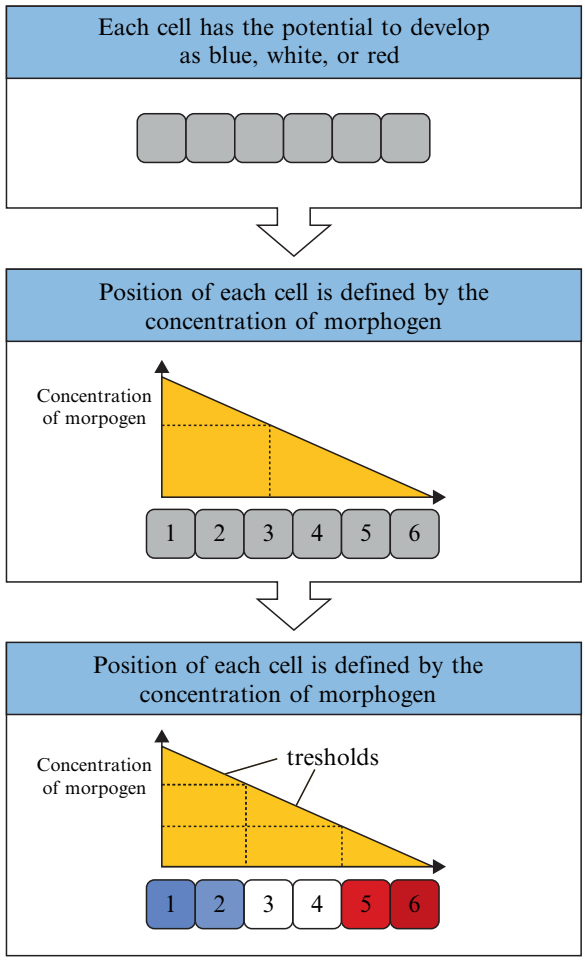

Fig. 13.1 Morphogens determine cell fate. Each cell has the potential to develop as red, white, or blue. A gradient of morphogen produced by a signaling center results in a different concentration at each cell, depending on the distance from the source. A threshold level determines cell fate and identity. A high concentration results in blue cells, medium concentration, in white, and low concentration, in red cells. (From [35], p. 24, Fig. 1.25, by permission of Oxford University Press)

13.1.2 Mechanisms of Morphogenesis

In the oldest sense of the word, a morphogen is a substance that is produced by cells and organizes a pattern by spreading to other cells [1]. Because morphogens are produced at one location, usually referred to as the signaling center, their concentration is thought to decline as a function of distance from the source. Thus, cells that are close to the signaling center will receive a high concentration of morphogen, while those further away receive lower doses. The hypothesis of smoothly declining gradients, originally proposed by Wolpert, assigns positional values to cells that are ultimately translated into cell fate determination (Fig. 13.1). The diffusing morphogen produces a gradient, which is superposed by other morphogen gradients. This results in cross-threshold values at which genes are turned on or off. This hypothesis has been supported by the identification of substances such as bicoid and decapentaplegic (Dpp) in *Drosophila* and, subsequently, polypeptides of the fibroblast growth factor (FGF), Wnt, Hedgehog, and transforming growth factor (TGF) families in vertebrates that act as intracellular or extracellular morphogens. During early *Drosophila* development morphogens can diffuse freely because the zygote nucleus undergoes a series of divisions in a common cytoplasm, the syncytial blastoderm. But how do morphogens move in the intercellular space? It is still not clear how gradients of

morphogens specify positional information, in particular the relative roles of morphogen diffusion and cell–cell interaction. Several models exist that try to explain how cell identity can be specified over long and short distances. Simple diffusion refers to randomly moving molecules that encounter little impediment from the tortuous intercellular spaces if concentration difference is the driving force of their movement. To direct the effect of a morphogen, evolution has developed intricate ways by modifying the spreading of freely moving molecules. Altering diffusivity allows the morphogen to accumulate to much higher levels near its source, paradoxically resulting in an increased range of action. Extracellular heparan sulfate proteoglycans, for example, promote the transport of *Drosophila* hedgehog protein and are essential for FGF signaling. Consequently, enzymes that change the local content of extracellular matrix heparan sulfate can have a great influence on hedgehog as well as FGF signaling. Other ways to modify diffusivity are lipid modifications; these occur in morphogens of the hedgehog and Wnt families. Morphogens may also be transported through cells by endocytotic vesicles, passing the morphogen on from one cell to the next. Receptor-mediated endocytosis and subsequent rapid degradation may serve to produce a sharp decline in protein concentration. Availability of morphogens may be altered by producing nonactive depot forms, as it is in the case of the transforming growth factors, which are activated upon proteolytic cleavage. Inhibitors may bind to morphogens, thus preventing their diffusion and/or binding to their cognate receptors. The BMP inhibitor Noggin, for example, completely inhibits BMP signaling, but is expressed at distinct sites that only partially overlap with BMP expression, thus directing and diversifying the signal (Fig. 13.2).

A wide variety of different signaling centers have been described that are essential for many developmental processes. In the *Drosophila* egg two "organizing centers" initiate anterior and posterior gradients, each forming its own structures at the poles and interacting with the other gradient to form the central portion of the embryo. Nüsslein–Volhard identified bicoid and hunchback as the proteins crucial for the anterior gradient and thus head and thorax formation, whereas nanos and caudal were found to form the posterior gradient and thus the abdominal segments [6, 7]. The anterior proteins inhibit the translations of the posterior proteins and vice versa, and as a result of this interaction a four-protein gradient is produced in the early embryo, which governs the first steps of *Drosophila* embryogenesis (Fig. 13.3). Signaling centers in vertebrates are, for example, the notochord and the floor plate, both producing the morphogen sonic hedgehog (Shh). As discussed below, different neurons develop in the neural tube depending on their distance from the signaling center and thus the concentration of the morphogen. A similar situation is seen in the limb, where Shh is expressed exclusively in the so-called zone of polarizing activity, a distinct region in the posterior part of the limb bud which controls the asymmetry of our limbs from the thumb to the little finger (Fig. 13.4) [30]. Depending on the distance from the center a pattern is built that determines which cells will finally develop into the individual digits. Duplications of this center to the anterior side result in mirror image duplications producing another set of digits. Many other signaling centers are known, and some of them are discussed below.

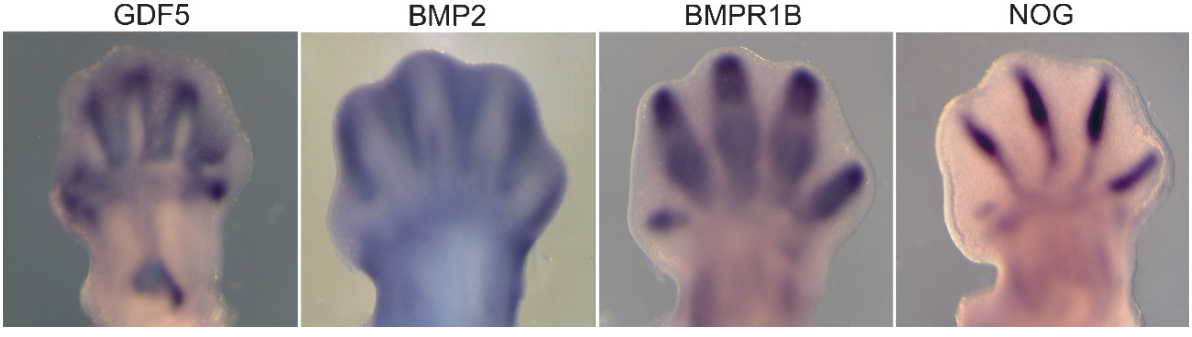

Fig. 13.2 Diverse expression patterns of morphogen, receptor, and inhibitor. Expression pattern of GDF5, BMP2, the receptor BMPR1B, and the inhibitor Noggin during development of the mouse digits. (Courtesy of P. Seemann)

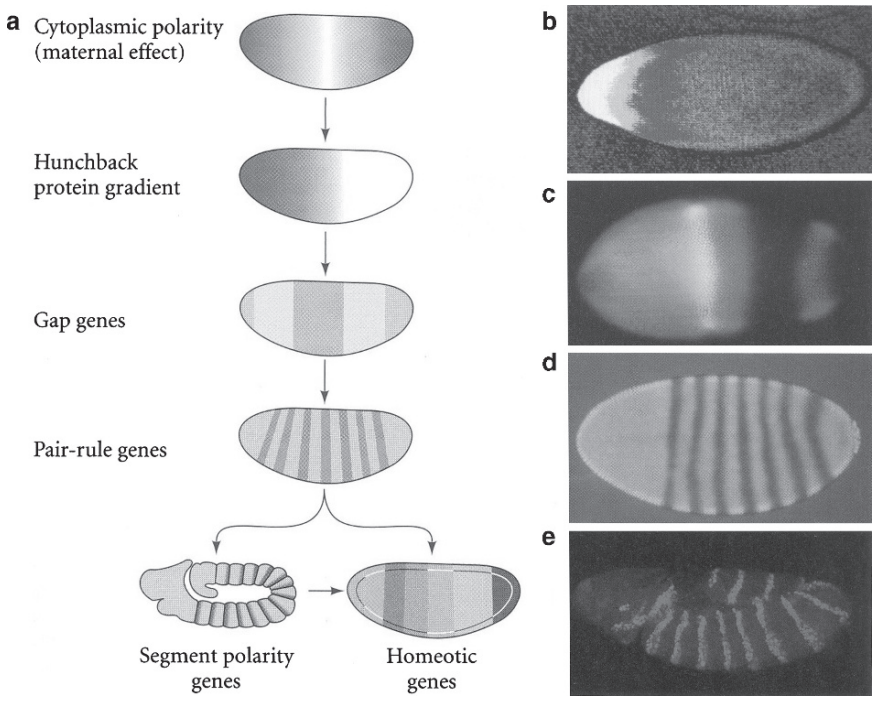

Fig. 13.3 (**a–e**) A generalized model of *Drosophila* pattern formation. (**a**) Anterior-posterior pattern is first generated by maternal effect genes that are located as sequestered mRNAs near the anterior tip (bicoid) and the posterior tip (nanos) of the unfertilized egg. After fertilization, the mRNAs are translated into proteins that can diffuse in the syncytial blastoderm, forming gradients that in turn activate Hunchback protein, which differentially activates gap genes, that define broad regions of the embryo. Gap genes activate pair-rule genes, giving the first indication of segmentation in the fly embryo. Pair-rule genes are expressed in a "zebra-stripe" pattern with an alternating pattern of vertical bands of cells expressing and not expressing a pair-rule gene. Together, these genes control the expression domains of the homeotic genes that define the identity of each segment. (**b**) Maternal effect genes. Bicoid protein concentration is highest at anterior tip (*bright yellow*) and diminishes towards the middle of the embryo (*red*). (**c**) Gap gene expression. Distribution of hunchback (*orange*) and Krüppel (*green*) overlap in the middle part (*yellow*). (**d**) Pair-rule genes. Pair-rule gene fushi tarazu forms seven stripes across the embryo. (**e**) Segment polarity genes. Expression ofengrailed dividing the embryo into a repeated series of segmental primordia along the anterior-posterior axis. (From [10], Fig. 9.17a–e)

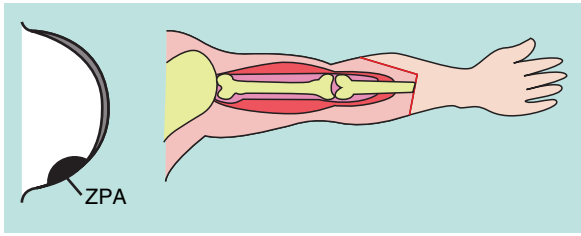

Fig. 13.4 Morphogen controls asymmetry of the limbs. Sonic hedgehog (*Shh*) is expressed in the posterior margin of the developing limb in a region called the zone of polarizing activity (ZPA). Digital identity from thumb to little finger is determined by the proximity to the signaling center and thus the concentration of Shh protein. (From [33], Fig. 2)

Morphogens need effectors, molecules that govern gene expression and by this means determine cell fate. In general this is not accomplished by single molecules but by an entire set of so-called transcription factors that bind to specific DNA sequences in the regulatory regions of target genes, thus resulting in an orchestration of gene expression. One example are the homeotic or *Hox* genes, originally identified in *Drosophila* for their ability to deliver positional identity to cells, meaning the information that tells each cell where it is in the embryo and how it has to behave to generate a regionally appropriate structure. Mutations in *Hox* genes produced a number of flies in which one body

Fig. 13.5 Ultrabithorax mutant. A mutation in the bithorax complex of the *Drosophila Hox* gene cluster results in the homeotic transformation of the posterior halteres (a balancing organ) into wings, resulting in a fly with two pairs of wings instead of one. (From [10], p. 284)

part developed in the likeness of another (called a homeotic transformation). In the mutant *Antennapedia* legs instead of antennae grow out of the head. In the mutant bithorax a second set of wings develops instead of the haltere, a small structure normally used by the fly to keep balance (Fig. 13.5). Molecular analysis has revealed that eight homeobox genes exist in *Drosophila* and that they are arranged in a cluster in the fly genome. Furthermore, the genes are expressed in an overlapping pattern along the head-to-tail axis, dividing the body into discrete zones (Fig. 13.6). The particular combination of genes expressed in each zone appeared to be essential for this the cell's positional information, since manipulating the genes either by mutating them or by overexpressing single *Hox* genes resulted in body part transformation [19, 21]. Very similar clusters of *Hox* genes are found in mammals, but here four clusters are present that are also expressed in an overlapping fashion, with most 3′ genes of a cluster being expressed most anterior and most 5′ in the dorsal region of the embryo (Fig. 13.6) [19]. Mutations in HOX genes in humans do not result in transformation of body parts, but instead lead to a variety of malformations involving the limbs and genitals (Fig. 13.7).

13.2 The Stages of Development

The development of animals actually begins before fertilization of the egg with the production by the female of substances that nourish and control the development of the zygote into a multicellular organism. After fertilization the zygote divides mitotically to produce the cells of the body, usually without much growth in overall size. In most invertebrates the resulting ball of cells is called a blastula, but in vertebrates the term morula is used. Even at this stage some cells have been determined to form specific tissues in the body. This was shown by experiments in which a piece of blastula is surgically excised and transplanted to a different position or onto another organism. In some cases the cells survive and continue their original path of development irrespective of their new environment. Thus, even before there is any visible distinction, cells are assigned to a specific fate. Cleavage divisions produce a hollow sphere of cells, the fluid-filled blastocele, which is called a blastocyst in mammals.

After the first rapid series of cell divisions, the blastula/morula undergoes a massive reorganization called gastrulation. This process converts an essentially nondescript sphere of cells into an organism with distinct cell layers, often called the primary germ layers. As originally studied in the sea urchin, gastrulation starts with an invagination of a subset of cells located at one side of the blastula into the blastocoel, the central cavity of the blastula. Through extensive cell movements the primary germ layers are formed. As a result, the embryo consists of the outer layer, the ectoderm, which produces the cells of the epidermis and the nervous system, the inner layer, the endoderm, which produces the lining of the digestive tube and its associated organs, and the middle layer, the mesoderm, which gives rise to several organs, including heart, kidney, gonads, and the skeleton. Although similar in principle, gastrulation follows different mechanisms in different species (Fig. 13.8). The major characteristic of avian and mammalian gastrulation is the primitive streak. This structure is visible as a thickening of the outer cell layer at the posterior region of the embryo caused by the ingression of mesodermal cells into the blastocoel and by migration of lateral cells towards the center. This streak marks the anterior–posterior axis of the embryo. A depression (primitive groove) forms within the streak, through which cells migrate into the blastocoel. Other migrating cells move between the two layers and form the mesoderm. At the anterior end of the primitive streak a regional thickening forms, called the primitive knot or Hensen's node. This structure is equivalent to the amphibian's blastopore, where migrating cells from the outside turn inward and travel along the inner surface of the outer cell sheets

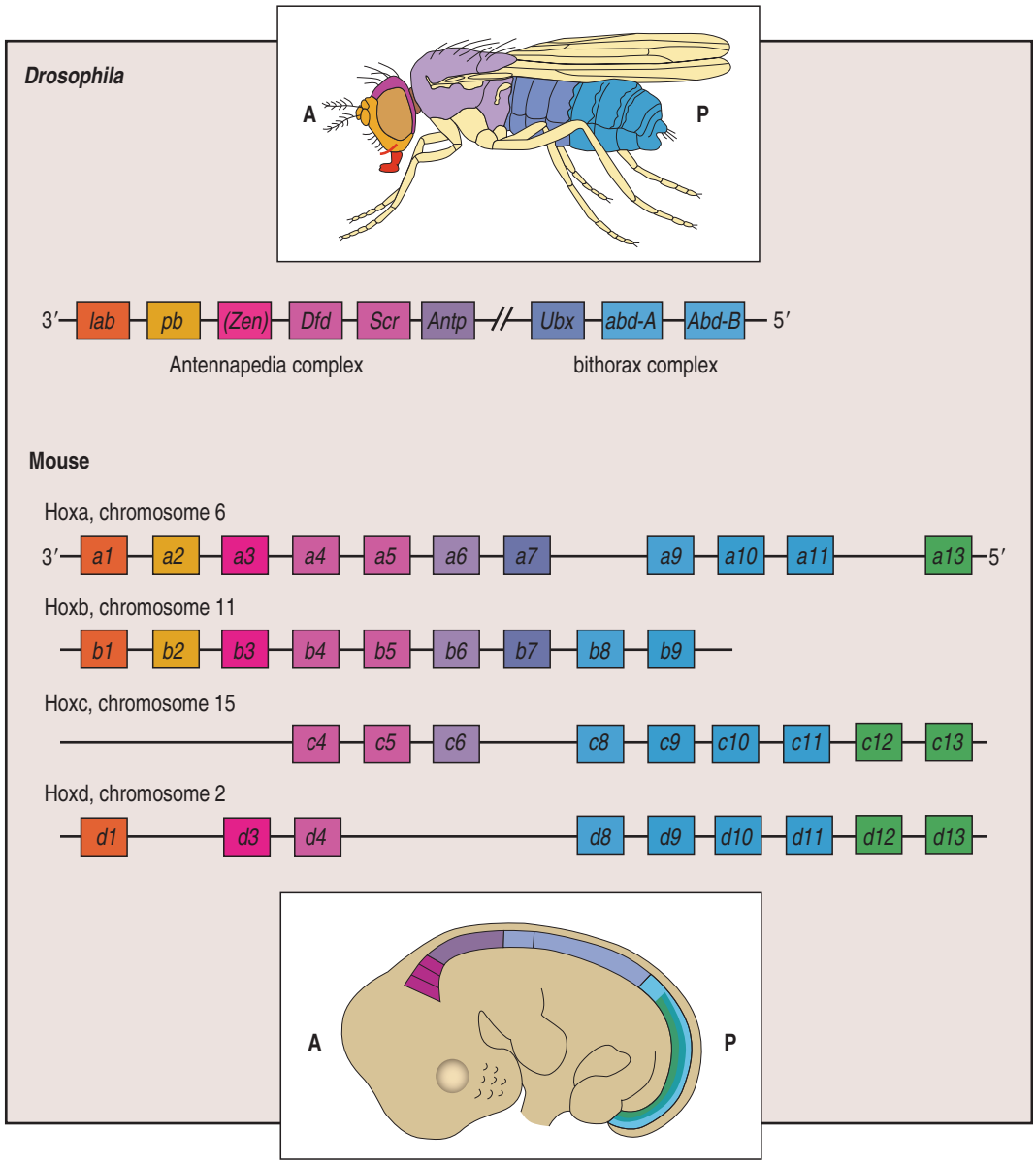

Fig. 13.6 *Hox* genes in *Drosophila* and mammals. *Hox* genes belong to a group of regulatory proteins (transcription factors) that control gene expression. In *Drosophila* there is one *Hox* gene cluster known as HOM-C, which consists of two distinct composites, the Antennapedia and the bithorax complex. In vertebrates *Hox* genes are arranged in four *Hox* clusters called A, B, C, and D, which are likely to have developed from genomic duplications of ancestral *Hox* clusters. In each cluster the order of the genes from 3′ to 5′ corresponds to the sequence in which they are expressed along the anterior-posterior axis of the embryo. Thus, the most 3′ located gene (*red*) is expressed first and furthest in the anterior direction, whereas the most 5′ (*blue/green*) is expressed last and furthest in the posterior direction (a phenomenon called spatial and temporal colinearity, respectively). Vertebrates have four *Hox* gene clusters (*Hox*A, -B, -C, -D), which originate from an ancestral cluster, possibly related to the single *Hox* cluster in the lancelet, a simple chordate. Genes that have arisen by duplication and divergence are referred to as paralogs, and the corresponding genes in each cluster (e.g., *Hoxa9*, *Hoxb9*, *Hoxc9*, *Hoxd9*) are known as a paralogous subgroups. Genes of a paralogous subgroup are more similar than genes within a cluster. (From [35], p. 156, Box 4A, by permission of Oxford University Press)

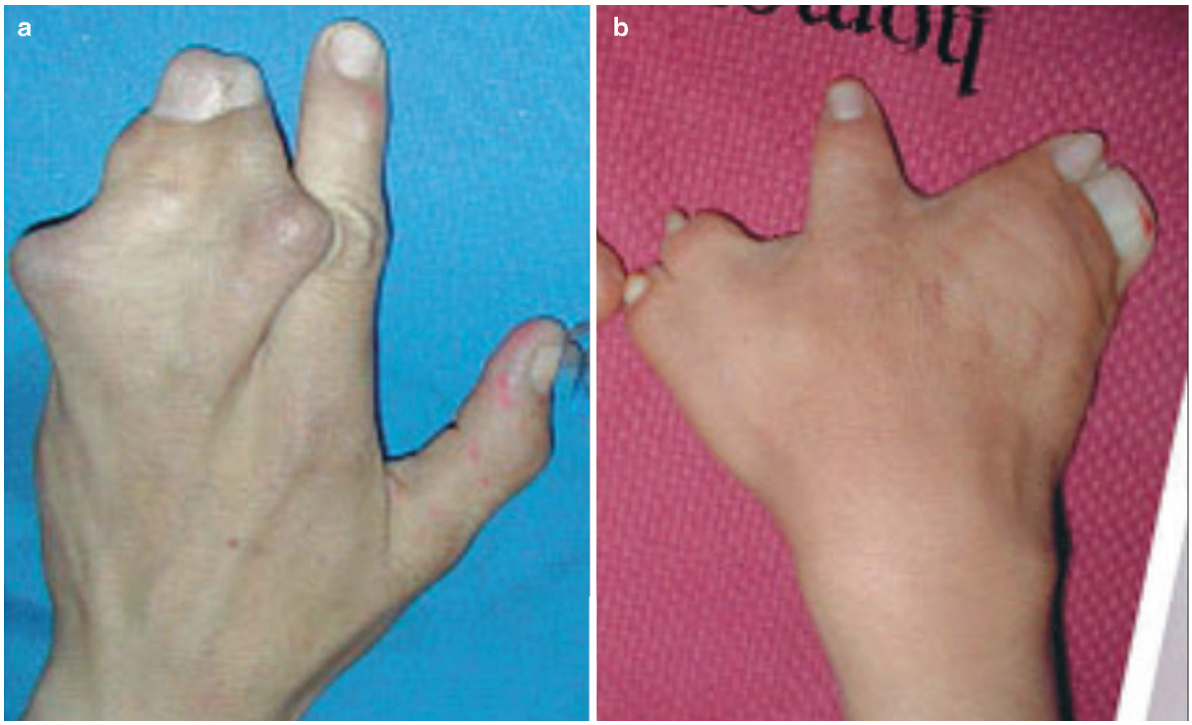

Fig. 13.7 (**a**, **b**) Synpolydactyly caused by a polyalanine expansion mutation in HOXD13. (**a**) Heterozygous mutations result in an additional finger between the third and fourth fingers and a syndactylous web between them. (**b**) Homozygous individual showing severe shortening of fingers, syndactyly, and polydactyly

(Fig. 13.9). Cells migrating through the primitive knot contribute to the foregut, the head mesoderm, and the notochord, whereas those that migrate through the lateral portions of the primive streak give rise to the other endodermal and mesodermal tissues. During this time a relatively small inner cell mass has become a bilaminar disk of ectoderm and endoderm, each with its own fluid-filled cavity, the amniotic sac, and the yolk sac.

In mammals early development follows the same principle, but not all cells contribute to the embryo, since a major proportion of cells is concerned with establishing tissues that are needed as a life support, namely the extraembryonic membranes and the placenta. Five to six days after conception the human blastocyst arrives in the uterus and attaches to the uterine wall (Fig. 13.10). The blastocyst now consists of an outer cell layer, the trophoblast and an inner layer, the embryoblast. The latter cells congregate at one end of the blastocele to form the inner cell mass. The trophoblast proliferates rapidly and differentiates into an inner layer of cytotrophoblast and an outer multinucleated layer, the syncytiotrophoblast, which starts to invade the uterine wall. During the second and third weeks of human development the invaded tissue becomes vacuolated and rapidly fills with blood. Chorionic villi grow into the vacuoles, bringing the maternal and embryonic blood supply into close contact and allowing the exchange of nutrients and waste products. The fully developed organ consiting of trophoblast tissue and the blood vessels is called the chorion. The chorion fuses with the uterine wall to create the placenta. Besides its role in the exchange of nutrients, the chorion has other important functions as an endocrine organ producing chorionic gonadotropin.

One of the early mesodermal derivatives is the circulatory system, which develops during the third week by vascular channels that arise in the splanchnic mesoderm lining the yolk sac; later a primitive heart that begins pumping by the end of the third week. The narrow connecting stalk that links the embryo to the trophoblast eventually forms the vessels of the umbilical cord. The embryo is now connected to its supply for

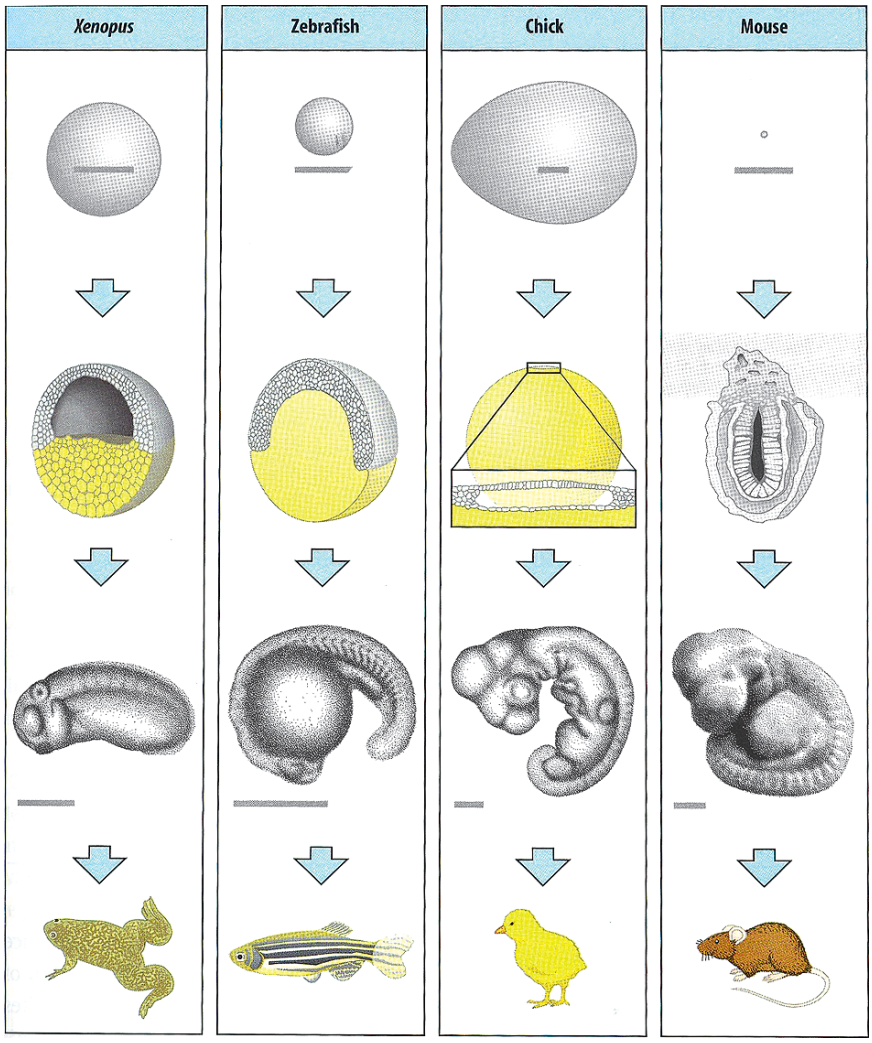

Fig. 13.8 Differences and similarities in development. Vertebrates show considerable differences at the start of their development. The size of the egg is very different (*scale bars* 1 mm except for the chicken egg: 10 mm). The *second row* shows a cross section through the blastula, with the egg yolk shown in *yellow*. At this time the mouse embryo has implanted into the uterine wall and has developed extraembryonic tissues. The embryo itself is the U-shaped structure in the *middle*. Thereafter, gastrulation commences and the embryos develop into polarized bodies consisting of ectoderm, mesoderm, and endoderm. In the following stage (*third row*) all embryos show a certain degree of similarity. The head has formed, and the neural tube, somites, and notochord are present. After this stage their development diverges again, giving rise to such diverse structures as wings, fins, and legs. (From [35], p. 91, Fig. 3.2, by permission of Oxford University Press)

nutrition, hormones and other essential substances, and the stage is set for the period of further patterning and major organogenesis. Early patterning events and the subsequent organ development are extremely complex processes that have been studied in a multitude of model systems. In this overview a few of these systems will be presented in an exemplary way without aiming at a full description of vertebrate organ development.

13.3 Formation of the Central Nervous System

Neurulation is the process by which the embryo forms a neural tube, the rudiment of the central nervous system. The formation of the neural tube is directly related to gastrulation, one of the most important processes in early development. The

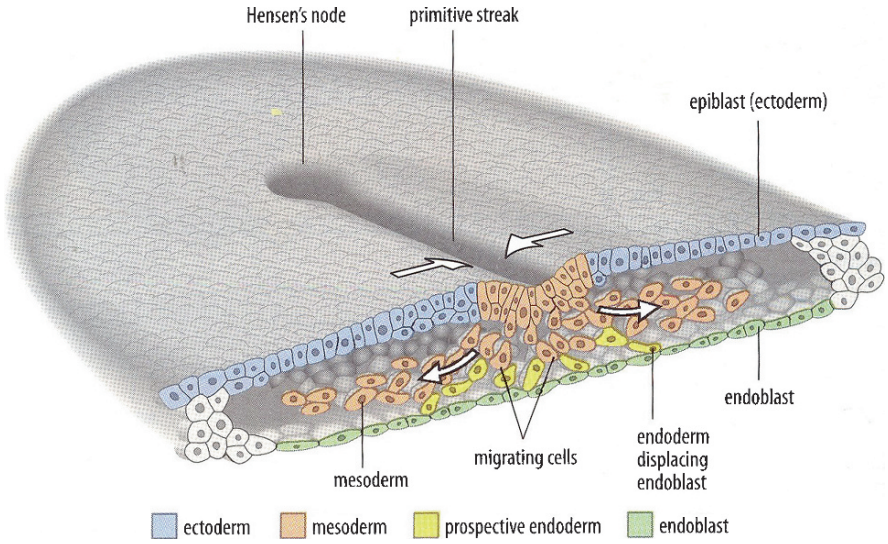

Fig. 13.9 Gastrulation in the chick embryo and Hensen's node. Cells migrate through the primitive streak into the interior of the blastoderm, where they give rise to the endoderm and the mesoderm. At the anterior end of the primitive streak an aggregation of cells known as Hensen's node can be seen. As the streak regresses, the node moves to the posterior end leaving behind the notochord and the first somites. (From [35], p. 102 Fig. 3.15, by permission of Oxford University Press)

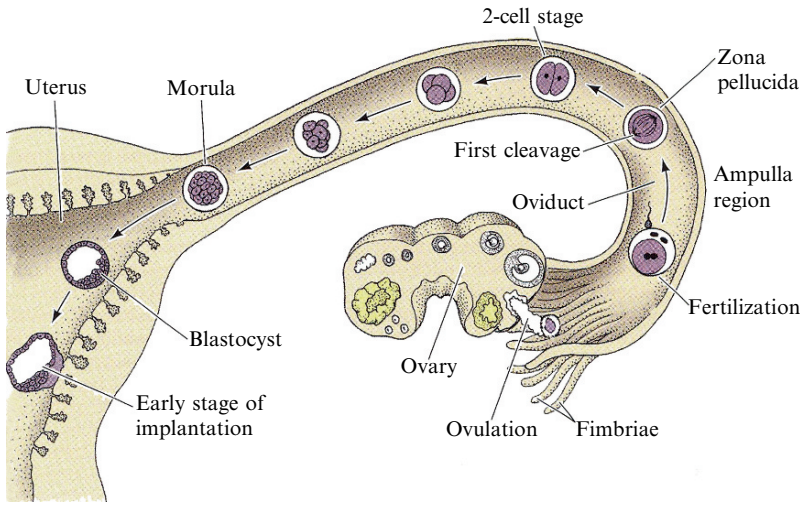

Fig. 13.10 Development of the human embryo from fertilization to implantation. Fertilization of the human oocyte takes place in the ampulla region of the oviduct, and the first cleavage occurs about a day later. The embryo keeps dividing at a slow rate as the cilia of the oviduct push the embryo towards the uterus. In contrast to most other embryos, mammalian blastomeres do not all divide at the same time, and they thus frequently contain odd numbers of cells. The zona pellucida surrounds the embryo and prevents it from attaching to the oviduct. Upon entry into the uterus the blastocyst escapes from the zona pellucida. In the mouse this is accomplished by digesting a small hole in it, through which the blastocyst "hatches." The uterine epithelium (endometrium) secretes a matrix that allows attachment of the blastocyst to the uterine wall. A cocktail of proteases secreted by the trophoblast enables the blastocyst to bury itself within the uterine wall. (From [10], p. 348, Fig. 11.27)

interaction between the dorsal mesoderm and its overlying ectoderm results in the formation of a hollow tube, which will differentiate into the brain and the spinal cord. The ectoderm folds at the most dorsal point, forming an outer epidermis and an inner neural tube. The two layers are connected by a specialized subset of cells, the neural crest. Folding ultimately results in the formation of the

spinal cord from the inner layer and closure of the epidermal layer, with the neural crest being situated in between. The neural tube closes as the paired neural folds are brought together at the dorsal midline. This process does not happen at one time point, but is an ongoing process that can best be observed in the chick embryo. While major regions of the neural tube may already be formed in the cephalic (head) region of an embryo, the caudal (tail) region may still be undergoing gastrulation, i.e., the migration of cells from the outer layer inside. Thus, tube formation progresses from head to tail in a zipper like format. In the cephalic region the wall of the tube is broad and thick and a series of swellings and constrictions define the future brain compartments. In those parts of the embryo that form the spinal cord, the neural tube remains a simple tube (Fig. 13.11).

In humans this process follows the same principle. However, the timing and the site of neural tube closure is different. In contrast to the process in the chick, human closure starts in the middle of the embryo and both, the anterior and the posterior neuropores, are open. Closure of the tube progresses from this initial site in both directions, toward the caudal and the cephalic part of the embryo. The cephalic part closes first, followed by the caudal part. Human malformations involving this process are common (Fig. 13.12). They present as spina bifida and anencephaly. In the latter condition there is failure of the neural plate fusion in the cephalic region while spina bifida is observed if the caudal part does not fuse. The reasons for this are complex, caused by genetic as well as environmental factors. Dietary factors such as cholesterol and folic acid appear to be important for normal development. It has been estimated that around 50% of neural tube defects can be prevented when pregnant women take supplemental folic acid. Cholesterol, on the other hand, appears to be necessary for the function of the Sonic hedgehog (Shh) signaling molecule, a morphogen that is essential for neural tube and brain development.

Neural crest cells start a long migration throughout the embryo, contributing to a vast number of tissues, including the bones of the skull, the teeth, the neuronal cells of the gut, and the heart. In humans several syndromes are known that are due to defects in neural crest migration and/or differentiation. For example,

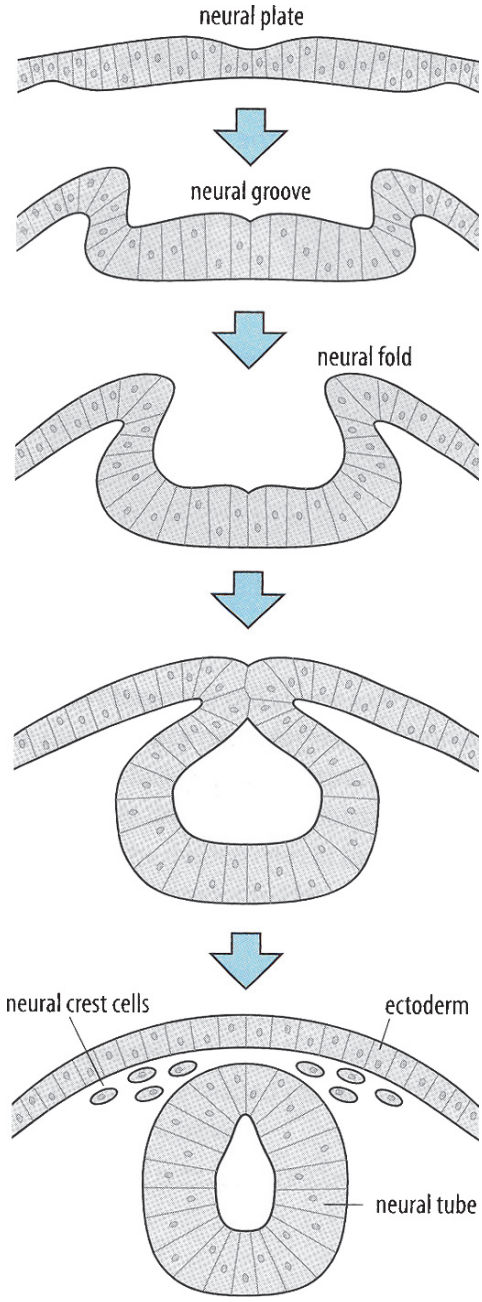

Fig 13.11 Development of the neural tube. Schematic cross sections through the early embryo are shown, representing different stages of neurulation from anterior (head, *top*) to posterior (tail, *bottom*). During neurulation the neural plate bends inwards, creating the neural groove with the neural folds at either side. With further development, the neural folds rise up, extend to the lateral side, and finally form a tube when they meet in the midline. This tube then detaches from the ectoderm, which becomes the epidermis. Neural crest cells which originate from the tip of the folds migrate away towards their distinct destinations. (From [35], p 283, Fig. 7.34, by permission of Oxford University Press)

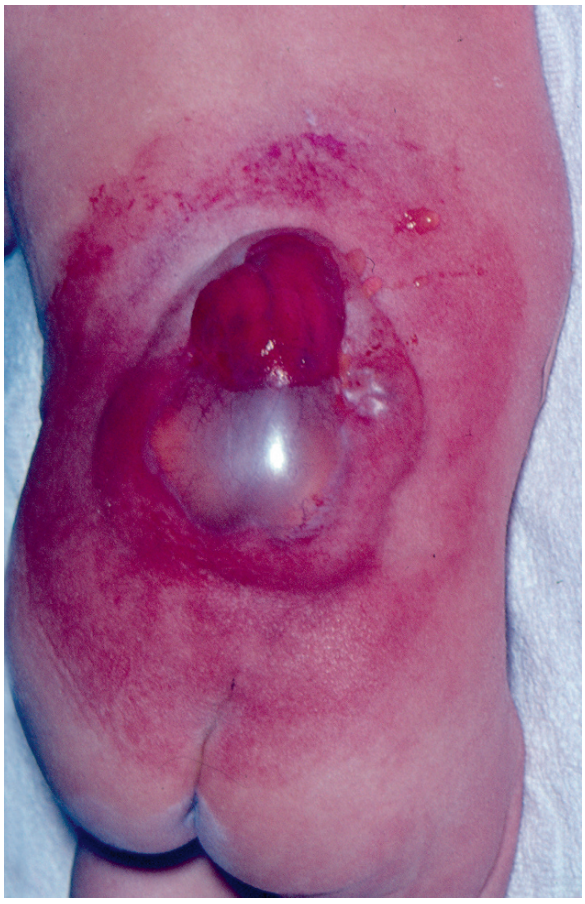

Fig. 13.12 Neural tube defect

mutations in PAX3 result in Waardenburg syndrome, a condition characterized by a specific facial appearance and unpigmented scalp hair. This pigmentation defect is believed to be due to missing neural crest cells, since these cells contribute to the pigment epithelium in the skin and hair.

While the posterior part of the tube is still being formed, the anterior or cephalic part undergoes drastic changes. In humans it subdivides into three primary vesicles, the forebrain (prosencephalon), the midbrain (mesencephalon), and the hindbrain (rhombencephalon). The anterior part of the prosencephalon bulges out laterally, building the two parts of the telencephalon, whereas the more caudal part of the prosencepahlon becomes the diencephalon. Furthermore, secondary bulges, the optic vesicles, extend laterally from each side of the prosencephalon. The mesencephalon does not become subdivided. Its lumen will eventually become the aqueduct connecting the ventricles. The rhomencephalon elongates and becomes subdivided into the metencephalon and the myelencephalon which eventually give rise to the cerebellum and the medulla oblongata, respectively (Fig. 13.13). During this process the early brain increases dramatically in size; however, this increase is primarily due to an increase in cavity size, and not to tissue growth. It has been speculated that increased fluid pressure inside the vesicle is the driving force for

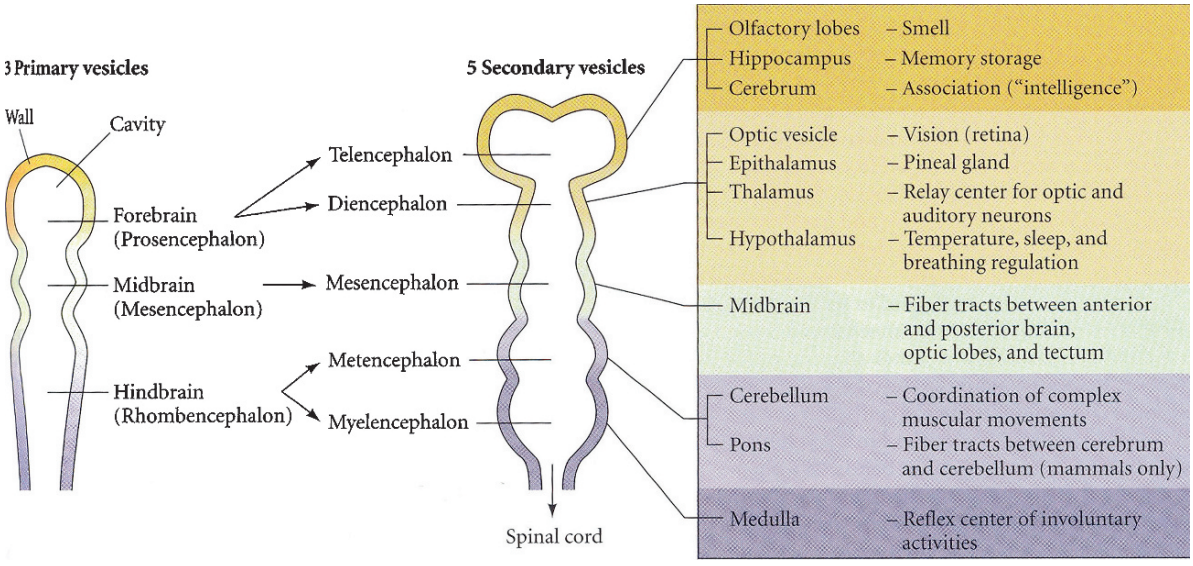

Fig. 13.13 Early human brain development. The three primitive brain vesicles, forebrain, midbrain, and hindbrain, are subdivided as shown on the *right*. These give rise to the adult brain derivatives listed in the *box*. (From [10], p. 381, Fig. 12.9)

this rapid expansion, since reduction of the pressure in chick embryos results in enlargement at a slower rate and the formation of fewer cells.

One important molecule that controls these early patterning events is Sonic hedgehog (Shh)[14]. Hedgehog was first discovered during a screen for mutants in *Drosophila melanogaster*, in which mutations in the single *Hh* gene that presents in this species give rise to an embryo that is covered in spiky cuticular processes called denticles, inspiring the "hedgehog" name. Shh, the vertebrate homologue of Hh, is a secreted molecule that undergoes extensive posttranslational modification including lipid modification, which influences the movement of Hh molecules between cells and its autocatalytic processing in an active and an inactive part. Through its receptor-patched (ptc) and the activation of the transcription factors Gli (cubitus interruptus (Ci) in *Drosophila*) Shh/Hh specifies neuronal identity over short and long distances. Shh's ability to specify identities as a function of its concentration is especially well illustrated by the vertrebrate neural tube, where it has a pivotal role in the generation of the diverse types of neurons that are required for the assembly of the spinal cord, the forebrain, and the retina. Shh patterns the neural tube from its two expression sites, the notochord and the floorplate, a triangular wedge of cells located at the ventral midline of the neural tube. The decreasing concentration of Shh from ventral to dorsal establishes distinct progenitor domains which prefigure and predict defined classes of neurons (Fig. 13.14). Without Shh this specification does not takes place and the neural tube consists mainly of dorsal type neurons and, for example, completely misses motorneurons.

How can one signal result in such diverse outcome? Expression of additional factors that modulate Shh signaling may result in a completely different effect. For example, regional differences to Shh signaling have a crucial role in the generation of complexity in the CNS of vertebrates. Shh is expressed in the developing brain in a narrow strip of cells in the vertebrate diencephalon, which is known as the zona limitans intrathalamica. Cells that lie posterior to this structure form the thalamus, whereas those located anterior give rise to the prethalamus. The target genes of *Shh*, *Ptch1* and *Nkx2.2* are expressed on both sides, but other important transcription factors (*Dlx2* on the anterior side and *Gbx2* on the posterior side) are expressed asymmetrically in response to *Shh*. Thus, the response to *Shh* may vary over time and space, further diversifying the response of cells to this important signal.

In humans, heterozygous mutations in SHH have been associated with variable forms of midline facial and brain dysmorphism, in particular holoprosencephaly (HPE) [27]. According to its clinical severity, three types of HPE have been delineated: alobar HPE, the

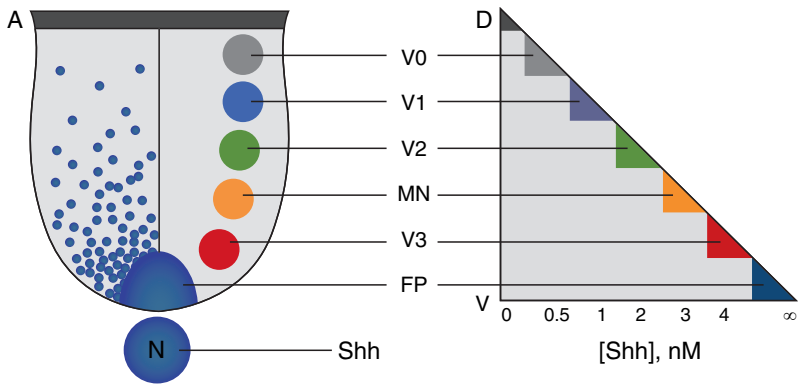

Fig 13.14 *Shh* and the control of spinal cord patterning. *Shh* expressed in the notochord and the floorplate defines the fate of ventral neural progenitors of the spinal cord. The progressively decreasing concentration of Shh protein regulates the expression domains of a series of transcription factors (as indicated by *color code*). *Shh* either represses or induces target genes at different concentration thresholds resulting in a complex pattern of expression combinations. The combinatorial expression of transcription factors in distinct domains determines the type of neuron that arises from each domain. (Reprinted from [15], by permission from Macmillan Publishers Ltd, *EMBO Reports*, copyright 2003)

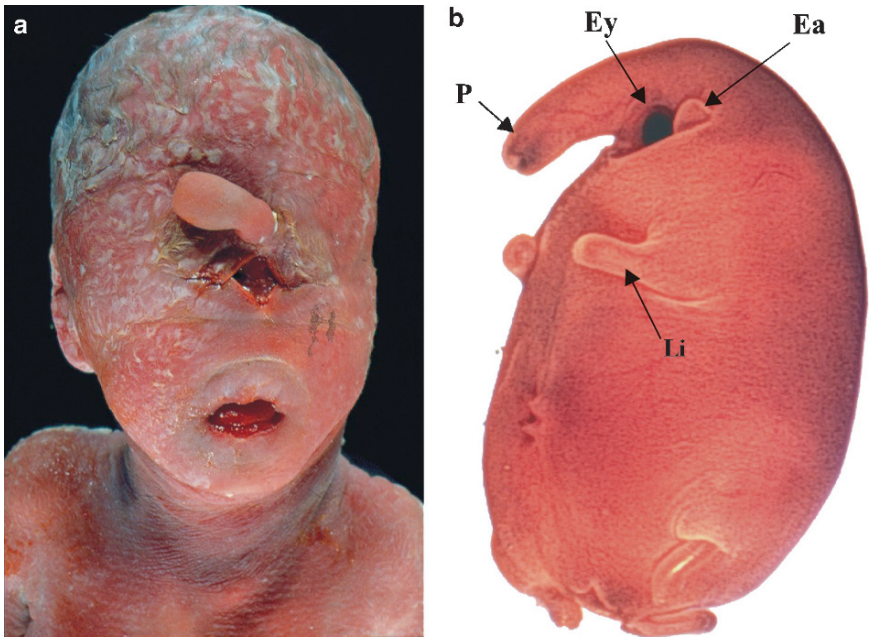

Fig. 13.15 (**a**, b) *Shh* regulates many aspects of development. (**a**) Patient with holoprosencephaly. Note nose-like structure in the middle with single eye underneath. (Courtesy of J. Kunze) (b) Mouse with inactivated *Shh*. Note single midline eye (cyclops) and nose-like structure above the eye (proboscis) (*P* Proboscis, *Ey* Eye, *Ea* Ear, *Li* Limb)

most severe form, is characterized by complete failure of the forebrain to divide into left and right hemispheres (Fig. 13.15). This form is associated with a single ventricle, and severe anomalies of the face, such as cyclopia, midline cleft, and single nostril are common. In semilobar HPE the interhemispheric fissure is partially present, whereas in lobar HPE the two hemispheres are separated but there is usually microcephaly. All three types, including completely asymtomatic carriers, can be observed within one family. This extreme variability raises the question of whether environmental factors can influence the outcome of this condition. Studies conducted in mammals and birds show that the severity of HPE defects correlates with the stage in which interruption in *Shh* signaling occurs [4]. Furthermore, levels of cholesterol may influence Shh processing and thus its activity. Inactivation of *Shh* in the mouse results in HPE and cyclopia, thus supporting the strong conservation of the pathway.

The initial patterning phase of the human cerebral cortex is paralleled and followed by three overlapping stages that form the structure of the brain. From weeks 5/6 to 6/20, stem cells deep in the forebrain proliferate and differentiate into young neurons or glial cells. During the second phase, between weeks 6/7 and 20/24, neurons migrate away from their place of origin in a radial fashion towards the pial surface, where each successive generation passes the previous one and settles in an inside-out pattern within the cortical plate. When migration is complete, the cortex consists of six layers of neurons that form discrete connections between the neurons and different parts of the CNS. Finally, from week 16 until well into postnatal life organization of the cortical layers takes place, a process associated with synaptogenesis and apoptosis. In this dynamic process more than one stage can occur simultaneously.

Developmental disorders accompanied by brain malformations are important causes of developmental delay, mental retardation, and epilepsy [12]. One of the best-described forms of human brain malformation is lissencephaly, a condition characterized by absent (agyria) or decreased (pachygyria) convolutions, producing a smooth cerebral surface attributable to loss of the folds of the brain, an abnormally thick cortex, and the loss of cortical lamination (Fig. 13.16). Subcortical band heterotopia is a related disorder that can be observed in different regions of the same brain, defining a spectrum of disorders with variable severity, all related to poor cortical architecture in the brain's development. Mutations in the *LIS1* gene cause lissencephaly [26].

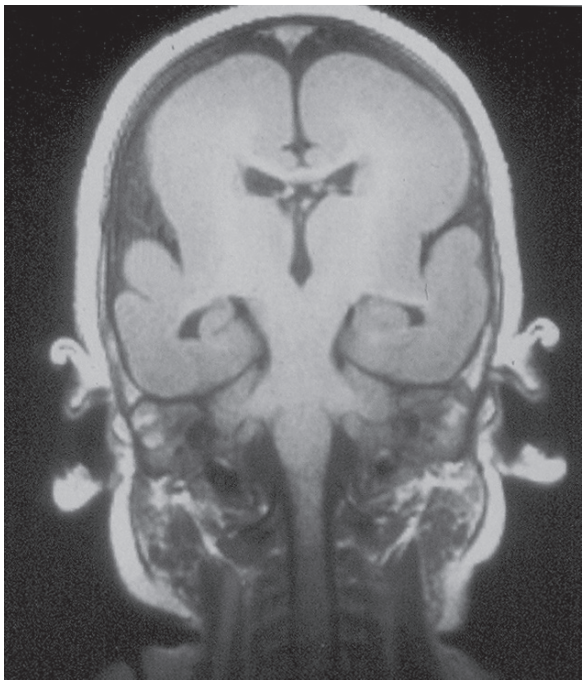

Fig. 13.16 MRI of patient with lissencephaly. Note "smooth" surface of brain owing to absence of gyri

Lis1 has been shown to influence neural progenitor proliferation and migration, possibly by interacting with microtubulin-binding proteins such as dynein and dynamitin. Loss of *Lis1* results in disruption of microtubule function, which interferes with forward translocation of the cell soma during migration and spindle orientation during mitosis. A second gene, DCX, causing an X-linked from of lissencephaly is likely to exert its effect on neuronal migration through its polymerization with microtubules.

Microcephaly vera means a reduction in brain size without marked brain malformation. Affected individuals have very small heads and a variable degree of mental retardation. Mutations in four genes have been shown to cause microcephaly vera; all are associated with the spindle poles of mitotic cells, and the mutations in them are consistent with a centrosomal mechanism in the control of cell division. Loss of microcephalin, for example, leads to premature chromosome condensation in G2 phase and delayed decondensation postmitosis through the condensin proteins. Interestingly, microcephalin has been implicated in human evolution including human brain size based on a strong positive selection in the human evolutionary lineage [8].

13.4 The Somites

Somites are a transient organizational structure of the developing embryo located on both sides of the neural tube consisting of epithelial cells with a periodic structure that originate from the paraxial mesoderm [11]. The formation of new somites and their detachment from the paraxial mesoderm has to occur in a highly ordered fashion simultaneously on both sides of the neural tube in the cranio-caudal direction. A time code for the formation and the budding of new somites is given by oscillations of cycling genes that lead to waves of notch-signaling sweeping up the paraxial mesoderm from the posterior to the anterior pole (Fig. 13.17). Beside this molecular clock, a stable gradient of *Fgf8* expression from the posterior to the anterior pole of the embryo allows a spatial coordination of somite border formation. Dll proteins are notch ligands that reside at the cell surface. Their differential expression determines both the size and the polarity of the somites. Any disturbance in this polarity results in abnormally spaced somites and in fusion of adjacent somites, as exemplified in the mouse mutant pudgy, which carries a mutation in *Dll3*. The phenotype observed in these mice resembles human vertebral malformations.

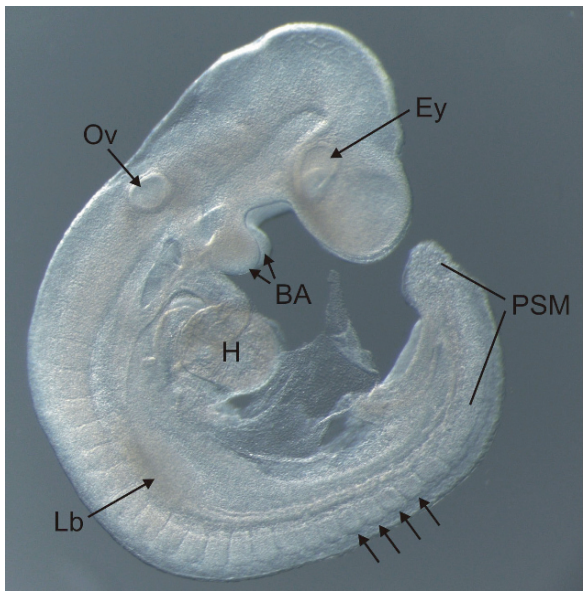

Fig. 13.17 Nine-day mouse embryo. Individual somites (*arrows*) can be seen, as can the paraxial mesoderm (PSM) (*Ey* Eye, *Ov* Otic vesicle, *Lb* Limb bud, *H* Heart, *BA* Brachial arches)

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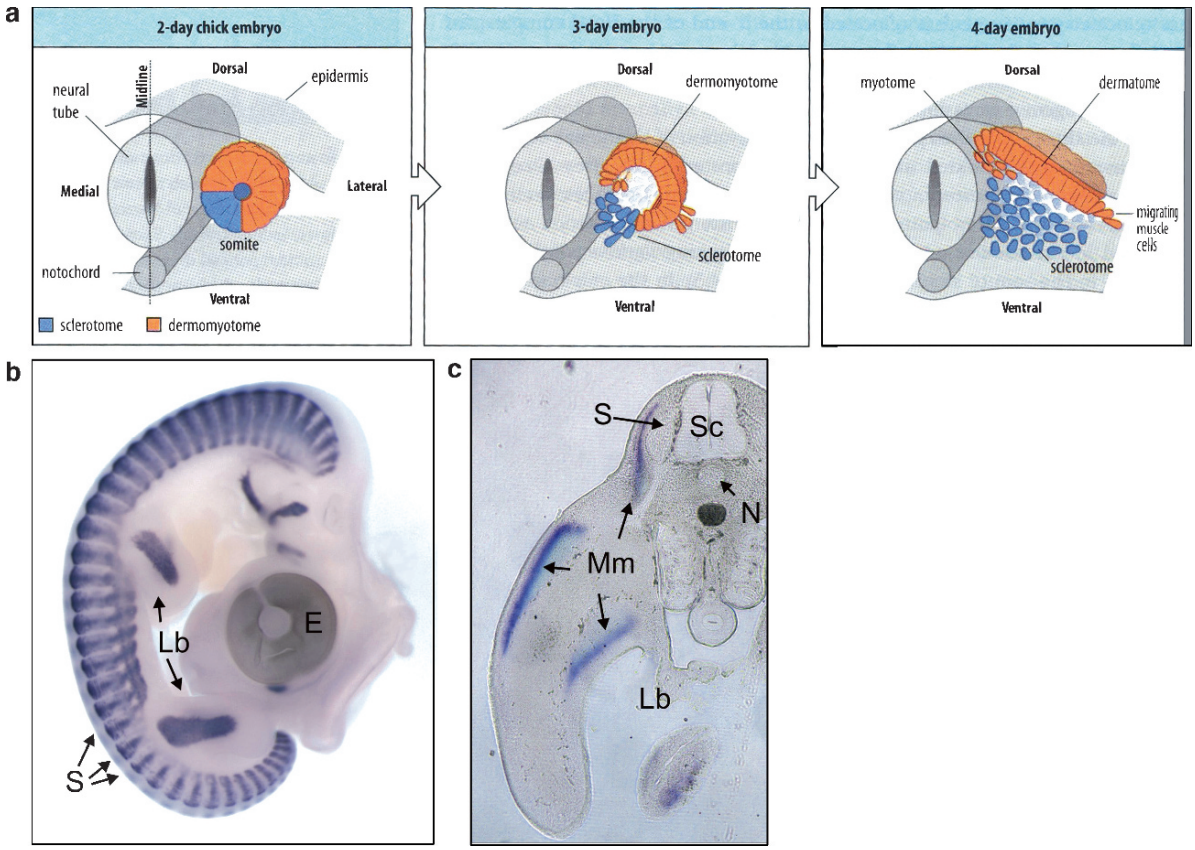

Fig. 13.18 (**a–c**) Development and fate of the somite. (**a**) Somites are transient structures that are formed periodically at each side of the neural tube from presomitic mesoderm. The medial quadrant of each somite gives rise to the sclerotome, whose cells will eventually form the vertebrae and the posterior part of the ribs. The rest of the somite forms the dermatome, which gives rise to the dorsal dermis and the myotome, the origin of all trunk and limb muscles. (**b**) Whole-mount in situ hybridization of a chick embryo showing expression of *MyoD*, a gene expressed during early muscle differentiation. Note periodic expression in somites and in the limb buds. (**c**) Cross section of embryo shown in **B** at anterior limb level. Staining shows migrating muscle cells (*Mm*) lateral to the somite (*S*) and in the limb bud (*Lb*) (*N* Notochord, *Sc* Spinal chord, *E* Eye. (From [35], p 164, Fig. 4.15, by permission of Oxford University Press)

As the somite moves rostrally, it matures and differentiates into the dermatome, the myotome, and the sclerotome (Fig. 13.18). A range of different tissues is generated from these structures. The dermatome originates from the central region of the dorsal layer of the somite (at this stage called the dermatomyotome). It generates the mesenchymal connective tissue of the back skin, the dermis. The myotome originates from the two most lateral portions of the somite. Its cells produce a layer of muscle precursor cells, the myoblasts, which form the epaxial muscles that will give rise to the intercostal and back musculature, and the hypaxial muscles of the body wall, limbs, and tongue. The epaxial part of the myotome is induced by signals from the neural tube (*Wnt1* and *Wnt3a*) and the floorplate (Shh), whereas the hypaxial region is induced by Wnt proteins from the epidermis and bone morphogenetic protein (BMP4) from the lateral plate mesoderm. The latter signal is likely to cause the migration of myoblasts away from the dorsal region into the body wall and the limbs. Further muscle development is established by a cascade of gene activation directed by a set of transcription factors, referred to as the myogenic regulatory factors, including *MyoD* and *Myf5*.

The sclerotome is the most medially located part of the somite and the primary origin of the axial skeleton. *Shh* is the major signal from the notochord that initiates and controls sclerotome formation. Transplantation of parts of the notochord (or *Shh*-producing cells) to other regions of the somite will result in the induction

of sclerotome cells at these sites. Sclerotome cells migrate towards the notochord and eventually surround it completely where they condense to form the anlage of the vertebrae. On each side of the neural tube the anterior part of a somite contributes to the caudal part of the vertebral body and the neural arch, whereas the posterior part of the next rostrally located somite is responsible for the rostral part of the vertebral body and the neural arch. It is evident that any disturbance of this process will result in abnormal anlagen of the vertebrae and thus in vertebral malformations. The notochord degenerates by apoptosis but sections of it remain and will eventually form parts of the intervertebral disk, the nucleus pulposus. In addition to the three major compartments of the somite, the sclerotome, the dermatome, and the myotome, two additional regions have been described. The syndetome is another layer of specified cells located between the myotome and the sclerotome. These cells are the precursors of cells that eventually form the tendons, which connect muscle to bone. The fifth compartment is present only in the trunk somites and contains cells that will form the vascular wall of the aorta and the intervertebral blood vessels.

Abnormalities of the ribs and/or vertebrae are a relatively common finding in human malformation syndromes. Those that primarily affect the axial skeleton are summarized under the term spondylocostal dysostoses (SCD) (Fig. 13.19). While the majority of these genetically heterogenous conditions remain unexplained, some types of SCD have been shown to be caused by mutations in members of the notch pathway such as DLL3 and NOTCH2, or in lunatic fringe (LFNG), a secreted protein necessary to maintain oscillation of Notch in the paraxial mesoderm. Affected individuals show a wide variety of vertebral malformations, including fusions and half-vertebrae.

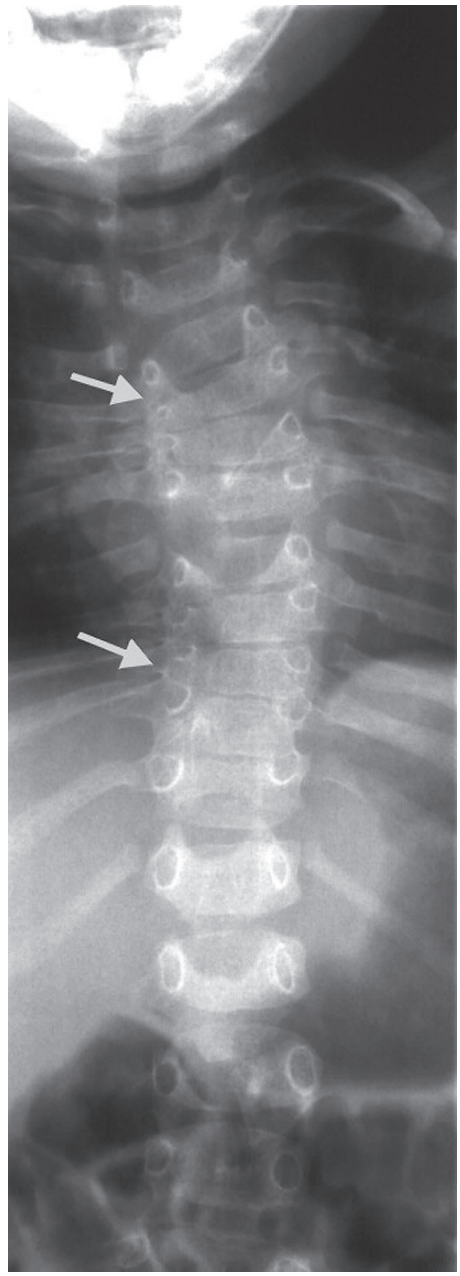

Fig. 13.19 Radiograph of a patient with spondylocostal dysostosis and mutation in DLL3. Organization of the vertebral column is chaotic and many vertebrae are fused or present only as halves

13.5 The Brachial Arches

The brachial or pharyngeal arches are another transient structure in the developing embryo that disappears along with the development of the neck and facial structures. They appear at about 3 – 4 weeks of human development as a series of bulges found on the lateral surface of the embryo. Four arches are found on each side, separated by clefts on the outside and so-called pharyngeal pouches on the inside (Fig. 13.17). Each pharyngeal arch is covered externally by ectoderm and internally by endoderm. The core of each arch contains neural crest cells, which surround a central group of mesodermal cells. Each cell type gives rise to a distinct set of tissues. The ectoderm generates the epidermal

covering of the arches, whereas the endoderm forms the endothelial lining of the pharynx, the thyroid, the parathyroid, and the thymus. The mesoderm forms both the musculature and the endothelial cells of the arch arteries. The neural crest cells generate the connective tissue of the neck and the skeletal tissues of each arch including Meckel's cartilage and the middle ear (stapes, incus, malleus). Although the arches represent repeated series of similar structures, they have been shown to give rise to distinct parts of the body. The most anterior arch forms the jaw, the malleus, and the incus of the middle ear, the second forms the stapes and the styloid ligament, and the third and fourth arches form the hyoid bone and the thyroid and the cricoid cartilage (Fig. 13.20).

During development, each arch must be patterned to receive its own identity and to be positioned within the embryo along the three axes. A number of mechanisms are known to achieve this, but it is clear that the endoderm plays a prominent role. It is the site where most of the signaling molecules are expressed and the endoderm has been shown to be responsible for the induction of particular arch components including cartilage formation and the precursors of the cranial sensory ganglia. Neural crest cells migrate from the neural tube into the arches. Transplantation studies in both amphibian and avian embryos have demonstrated that crest cells acquire positional information when they are within the neural tube and transfer this information into patterning cues when they have reached the arches. Crest cells migrate in three streams separated by two regions, rhombomeres 3 and 5, which are basically depleted of crest cells. BMP4 have been shown to induce cell death of crest cells in these rhombomeres, while the flanking cells are protected by expression of the BMP inhibitor Noggin. However, other studies point to roles of the mesoderm and the surface ectoderm in patterning the arches, and there is evidence that *Hox* genes play the major part in this process. Inactivation of *Hoxa2*, for example, results in homeotic transformation of elements derived from the second arch into first arch derivatives, including partial duplications of Meckel's cartilage and the ossification centers of the middle ear bones.

The combination of hearing loss (sensorineural and/or conductive), preauricular pits, branchial fistulas, and renal dysplasia characterizes the branchio-oto-renal syndrome. With the exception of the renal problem, these anomalies are considered defects of the brachial arches, and EYA1, the gene mutated in this condition, is expressed in the neural cells of the first arch and in the second, third, and fourth pharyngeal pouches and clefts. Defects of the brachial arches result in

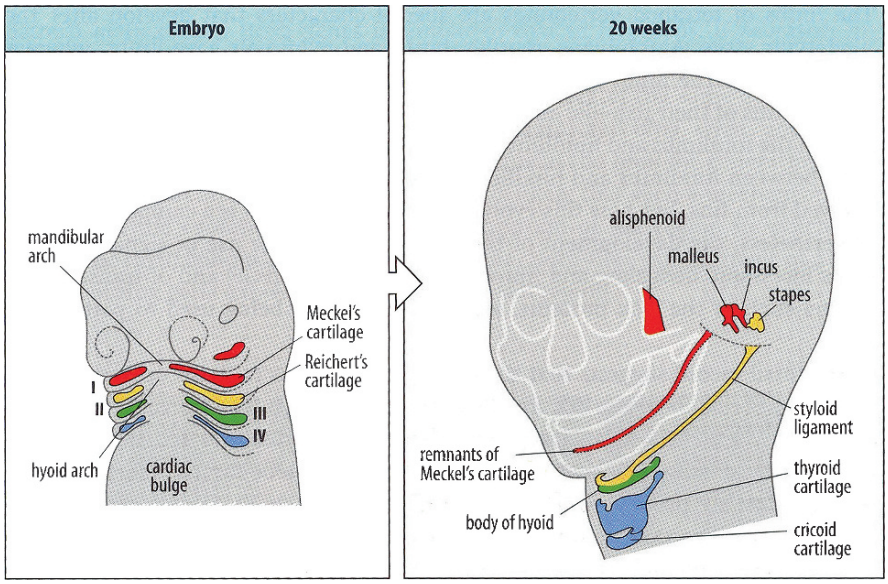

Fig. 13.20 Fate of brachial arch cartilage in humans. Cells from the brachial arches give rise to the three auditory vesicles, the hyoid, and the pharyngeal skeleton. The fate of the various elements is shown by the *color coding* (From [35], p. 502, Fig. 14.6, by permission of Oxford University Press)

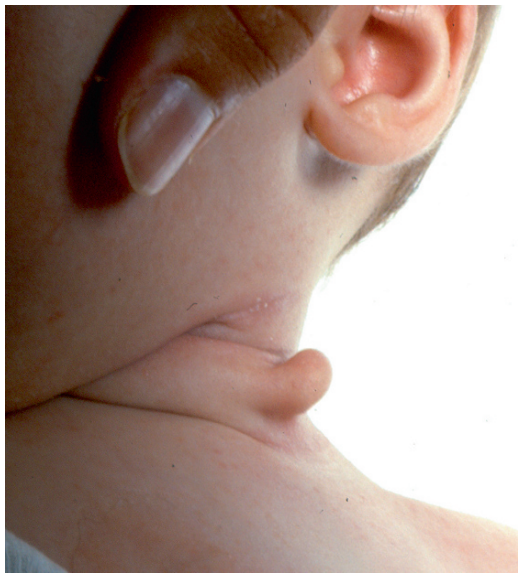

Fig. 13.21 Patient with brachial cyst. (Courtesy of J. Kunze [34], p. 8, Table 4.15)

malformations of the neck, such as brachial fistulas or cysts (Fig. 13.21).

13.6 Development of the Limbs

The limb skeleton originates from the lateral plate mesoderm. All other limb structures, including muscles, nerves, and vasculature, originate from the somitic mesoderm. Outgrowth of the limb bud is the result of a series of interactions between the mesoderm and the overlying ectoderm. As the limb bud grows out, mesenchymal cells begin to differentiate to form the various tissues of the limb in a proximo-distal sequence with structures being laid down progressively from a region of undifferentiated mesenchymal cells at the tip of the limb bud, known as the progress zone. The positional identity, and thus differentiation, of each cell is controlled by a three-dimensional coordinate system consisting of the dorso-ventral, the proximo-distal and the antero-posterior axes. Each axis in controlled by a particular set of signaling molecules/pathways produced by a defined population of cells (signaling centers). The combination of these signals informs the undifferentiated cells in the mesenchyme about their position and their fate in order to form the appropriate structures. Three signaling regions that convey this information have been identified: the apical ectodermal ridge (AER), mediating limb bud outgrowth (proximo-distal axis), ectoderm covering the dorsal sides of the bud governing dorso-ventral pattern, and the zone of polarizing activity (ZPA) controlling antero-posterior pattern (Fig. 13.22). Many of the signaling molecules that are produced by these signaling centers have been identified and characterized and intracellular signaling transduction pathways are being unraveled [32]. Furthermore, mutations in human limb malformations are being identified that help to understand the normal and abnormal mechanisms of limb development [28].

The AER is an anatomical structure consisting of densely packed ectodermal cells located at the very tip of the limb bud. Several different fibroblast growth factors (FGFs) are expressed and secreted by the AER and have been shown to be both, essential and sufficient to initiate and control outgrowth of the limb. FGF signaling is conveyed through the FGF receptors, which are expressed in the underlying mesenchyme. Experimental removal of the AER results in an arrest of limb outgrowth and thus truncations of the limb, depending on the time point of the intervention (Fig. 13.23). Mutations that result in AER loss have a similar outcome, leading to various degrees of limb defects. In humans this mechanism is associated with the ectrodactyly phenotype. This severe limb malformation is characterized by variable degrees of central defects of the digits resulting in cleft hands/feet, or, in the most severe cases, adactyly/monodactyly (Fig. 13.24). Ectrodacyly is genetically heterogeneous, but mutations in TP63L appear to be the most common cause.

The ZPA is a region of mesenchyme located at the posterior limb bud margin. Sonic hedgehog (*Shh*) is expressed in this region and has been shown to be the main mediator of antero-posterior patterning. Implantation of *Shh*-expressing cells can rescue surgical ZPA removal, and expression from the anterior margin of the limb bud results in the formation an anterior ZPA with subsequent mirror duplication of the entire autopod (Fig. 13.25). The restricted expression of *Shh* in the anterior part of the limb bud appears to be regulated by a conserved region approx. 1 Mb away from the *Shh* promotor known as the ZRS. Mutations in ZRS result in polydactyly in humans, mice, and cats by dysregulating *Shh* expression in the limb bud, thus creating an additional anterior *Shh* expression domain

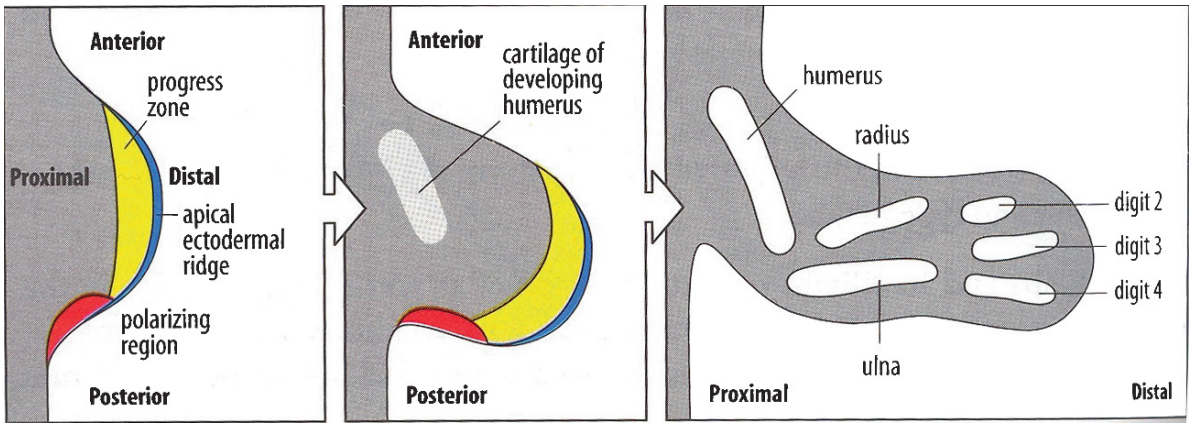

Fig. 13.22 Development of the chick wing with its centers of differentiation and signaling. Cells in the limb bud receive signals from the zone of polarizing activity (ZPA) localized at the posterior margin of the limb, and the apical epidermal ridge (AER), a specialized anatomical structure at the very tip of the bud. The ZPA specifies position along the anterior-posterior axis, whereas the AER controls the proximo-distal axis. Signals from the AER keep cells in the so-called progress zone in an undifferentiated and proliferative state. Once they leave this zone they start to differentiate, giving rise to the mesodermal derivatives of the limb, e.g., the skeleton. Thus, the individual skeletal elements are laid down in a sequential order from proximal to distal. (From [35], p. 142, Fig. 9.6, by permission of Oxford University Press)

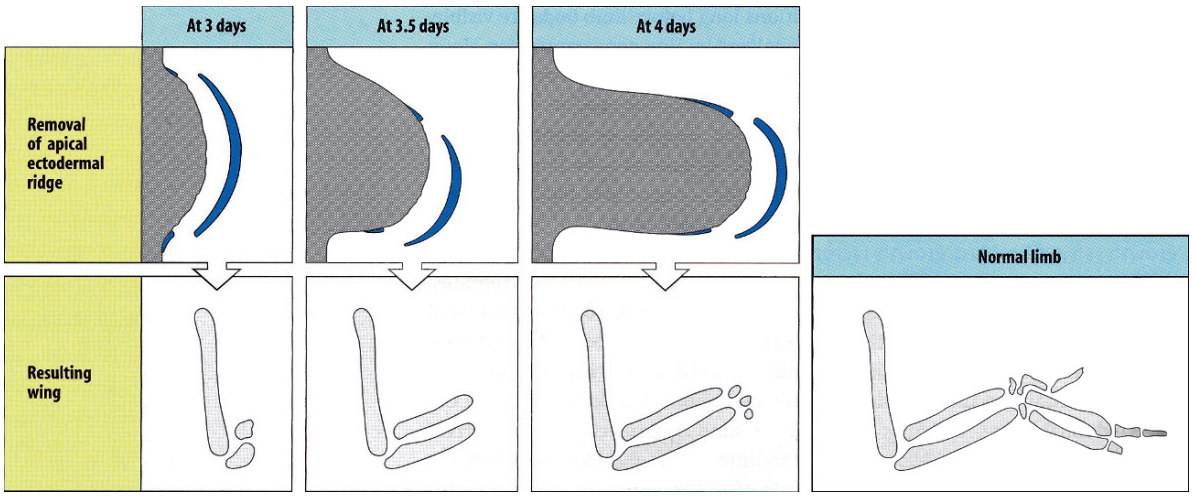

Fig. 13.23 Removal of the AER results in arrest of limb outgrowth and truncation. Surgical removal of the AER results in limb truncations at different levels, depending on the time point of removal. (From [35], p. 341, Fig. 9.7, by permission of Oxford University Press)

(Fig. 13.25b–d). Thus, this regulatory mutation has the same effect as the ZPA transplantation, i.e., activation of ectopic anterior *Shh* expression and subsequent mirror image duplications.

Under the influence of *Shh* the zinc finger transcription factor *Gli3* is converted into the activating form *Gli3A*, whereas otherwise the repressor form *Gli3R* predominates, which in a negative feedback downregulates *Shh*. By this mechanism *Gli3* expression is much stronger in the anterior than in the posterior limb bud, where *Shh* levels are high, thus creating an anteroposterior gradient that has been shown to be important for limb patterning. Mutations in GLI3 result in the Greig and Pallister–Hall syndromes, two conditions characterized by various degrees of polydactyly associated with midline malformations and benign tumors (hamartomas) (Fig. 13.26). In mice it has been shown that *Gli3* deficiency leads to ectopic expression of

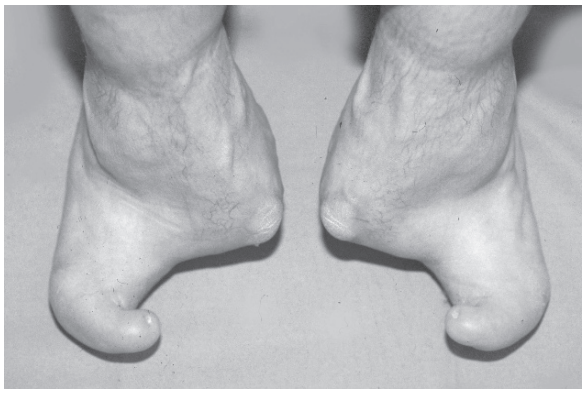

Fig. 13.24 Ectrodactyly associated with mutation in TP63L

Shh in the anterior margin of the limb bud in addition to the normal expression of *Shh* in the ZPA. This results in a double dose of *Shh* and a second ZPA, thus explaining the polydactyly.

A member of the Wnt family of growth factors, Wnt7a, has been shown to be important in dorso-ventral patterning. Expression of Wnt7a in the dorsal ectoderm of the limb bud up-regulates Lmx1b, which belongs to the family of LIM homeodomain transcription factors and forms a dorso-ventral gradient. This close functional relationship explains why Wnt7a-deficient and Lmx1b-deficient mice both develop autopods with a double ventral phenotype. Mutations in

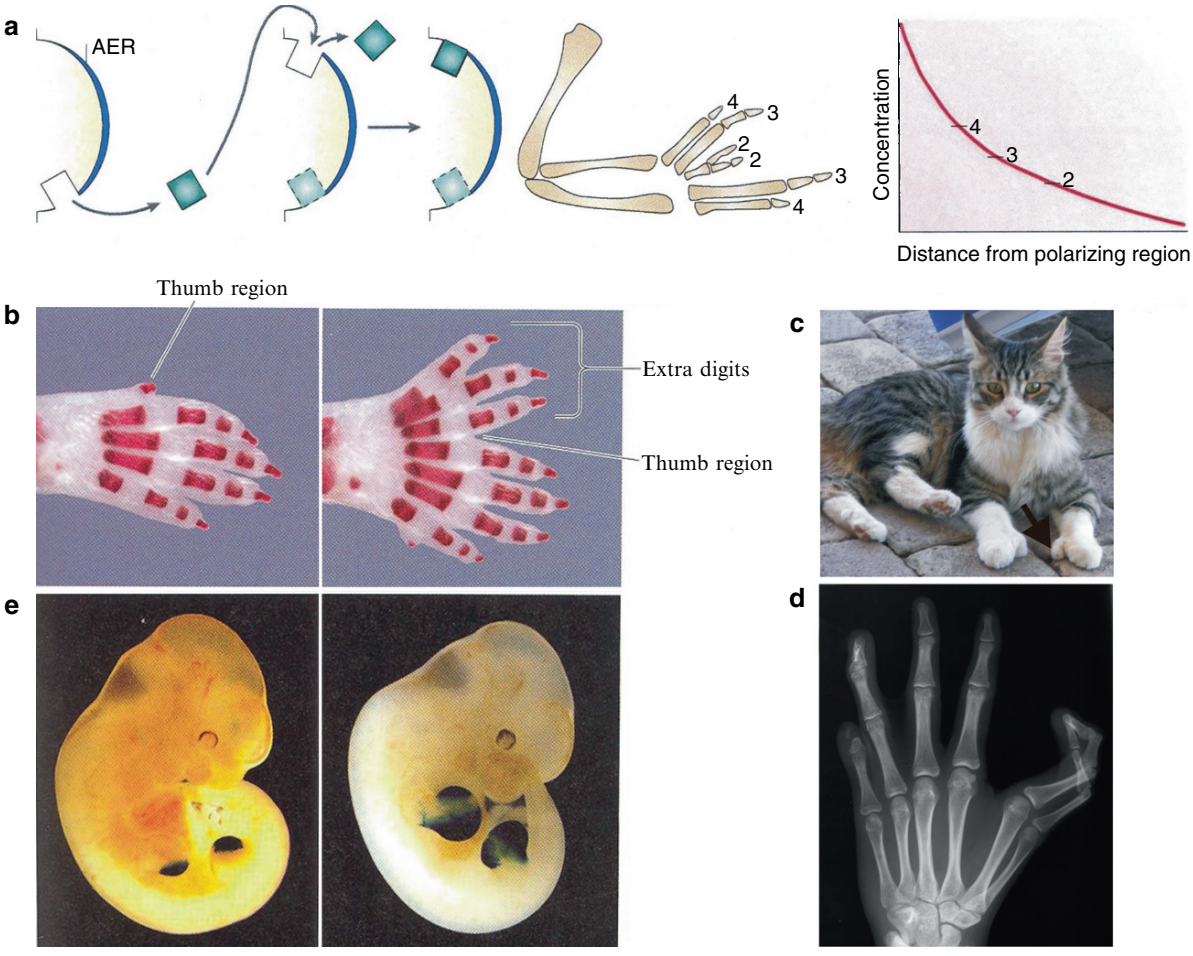

Fig. 13.25 (**a–e**) Digit duplications by AER duplication. (**a**) If an AER is grafted from a donor limb to the anterior region of a recipient a mirror image duplication of the digits is observed. This is due to a double dose of Shh morphogen from both the anterior and the posterior side. Since the distance from the source specifies digit identity, two posterior sides are created. (Reprinted from [32], by permission from Macmillan Publishers Ltd, *Nature Reviews Molecular Cell Biology*, copyright 2005) (**b–d**) Mutations in the regulatory region of the Shh gene in mouse (**b**), cats (**c**), and humans (**d**) result in polydactyly due to ectopic *Shh* expression at the anterior margin of the limb bud. (**e**) Transgenic mouse embryos stage E 12.5 expressing *LacZ* (*blue staining*) under the control of wt (*left*) or mutant (*right*) *Shh* limb regulatory region. Note expression restricted to the posterior limb in embryos expressing the wt construct and expanded expression domain in the mutant. ((**b**, **e**) from [10], p. 517, Fig. 16.17a–d)

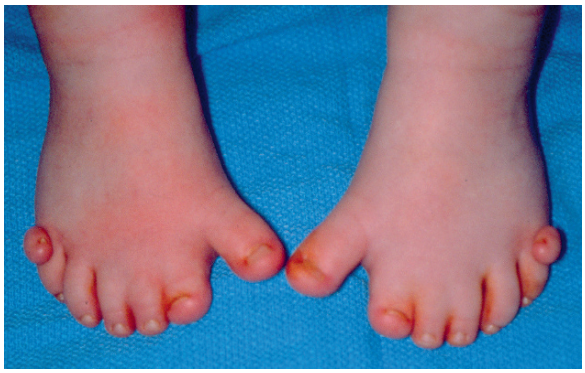

Fig. 13.26 Polydactyly in Pallister–Hall syndrome caused by mutation in GLI3

LMX1B in humans result in nail-patella syndrome, a condition characterized by absent/hypoplastic patellae and dysplastic nails. Both structures represent the dorsal part of the limb, and thus nail-patella syndrome represents a "ventralizing" phenotype. Mutations in WNT7A cause a range of phenotypes, again associated with loss of dorsal structures (nails, patellae), but in most severe cases these mutations are associated with phocomelia, probably because of a complete breakdown of all three signaling centers.

Hox genes from the 5′ region of the A- and D-clusters show characteristic stage-dependent expression patterns that determine the shape and identity of the limb skeletal elements. They are expressed in overlapping patterns, with most 5′ genes having the smallest, the most posterior, and the most distal expression domain. During later stages of development these domains change in a very dynamic way, resulting in an expression of the most 5′ *Hoxd* gene, *Hoxd13* over the entire hand/foot plate. Gene inactivation experiments in the mouse have shown that these domains also correspond to later anatomical regions (Fig. 13.27) in that loss of *Hoxd11* and *Hoxa11* results in absence of radius/ulna and tibia/fibula, whereas loss of *Hoxd11*, *-12*, *-13*, and *Hoxa13* results in a severe reduction of hands/feet. This pattern of expression is probably achieved by a repression mechanism that is gradually released during development located in a chromosomal region upstream of *Hoxd13*. Mutations in *Hox* genes result in limb malformations as illustrated by synpolydactyly, a condition characterized by fusion of fingers (syndactyly) together with an additional finger in the syndactylous web (polydactyly) (see also Fig. 13.7).

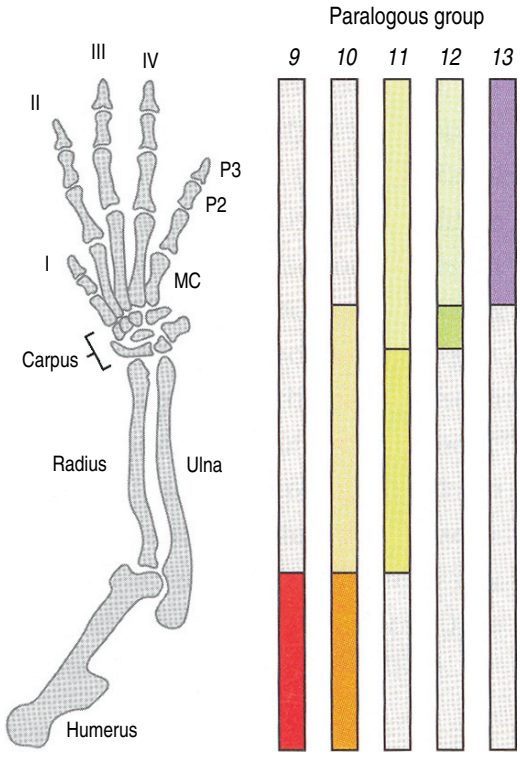

Fig. 13.27 (**a, b**) *Hox* gene patterning of the forelimb. (**a**) *Hox* genes of one paralogous group are expressed in distinct domains of the developing limb that correspond to adult skeletal elements. Whereas *Hoxd13* is exclusively influences patterning of the hand (autopod), *Hoxd11* together with *Hoxd10* are important for pattering of radius and ulna (zeugopod). *Hoxd9* and *Hoxd10* function together to pattern the humerus (stylopod). (From [35], p. 353, Fig. 9.18). (**b**) *Hoxd13* expression in the limb buds and the genital ridge of a mouse embryos stage E12.5. By permission of Oxford University Press

13.7 Development of the Circulatory System

Presumptive heart cells originate in the early primitive streak and form two groups of cells lateral to Hensen's node. At the same time, angiogenic clusters form that contain the first blood cells. These are soon surrounded by a double-walled tube consisting of the inner endocardium and the outer epimyocardium, which will form the endocardium, i.e., the inner lining of the heart and the heart muscles, respectively. As the foregut is closed, the two tubes are brought together and fuse to a single pumping chamber. In humans this occurs at 3 weeks of gestation. By 5 weeks the heart has developed into a two-chambered tube with one atrium and one chamber. The partitioning into the two chambers is

accomplished by cells that migrate into a hyaluronate-rich structure, the endocardial cushion, that is later located between the ventricles and the atria. Meanwhile the atrium is partitioned by the development of two septa that grow ventrally towards the endocardial cushion. These septa stay open, thus providing blood flow from the right to the left atrium. In the 7th week of human development the ventricles begin to be separated by the growth of the ventricular septum towards the endocardial cushion [31]. Figure 13.28 shows a schematic of human heart development.

For a proper orientation of the pulmonary (right) and systemic (left) ventricles and for the alignment of the heart chambers with the vasculature the linear heart tube undergoes rightward looping [25]. The direction of cardiac looping is determined by an asymmetric axial signaling system that also affects the position of the lungs, liver, spleen and gut. Before organ formation begins, this signaling cascade directs the asymmetrical expression of Sonic hedgehog (*Shh*) and Nodal, a member of the TGFβ family. In humans mirror image reversal of right–left asymmetry (situs inversus) is often associated with normal organogenesis, but discordance of cardiac and visceral asymmetry (situs ambiguus, also called heterotaxy) is associated with malformations of the heart and other organs. In the latter condition asymmetry in structure and placement of organs still develops, but, owing to the lack of definitive positional information, this happens on a stochastic basis. Cardiac defects typically occurring with situs ambiguus include, but are not limited to, atrial septal defects, ventricular septal defects, transposition of the great arteries, double-outlet right ventricle, anomalous venous return, and aortic arch anomalies. But what determines right–left asymmetry in the first place? Evidence that ciliary function might be involved came from studies of Kartagener syndrome, a condition characterized by situs inversus together with recurrent pulmonary infections attributable to defects in cilia function. Elegant experiments performed with video microscopy have shown that motile cilia are present in the center of the mouse node that propagate directional fluid flow. In addition, it was shown that this flow is abnormal in several mouse mutants with laterality

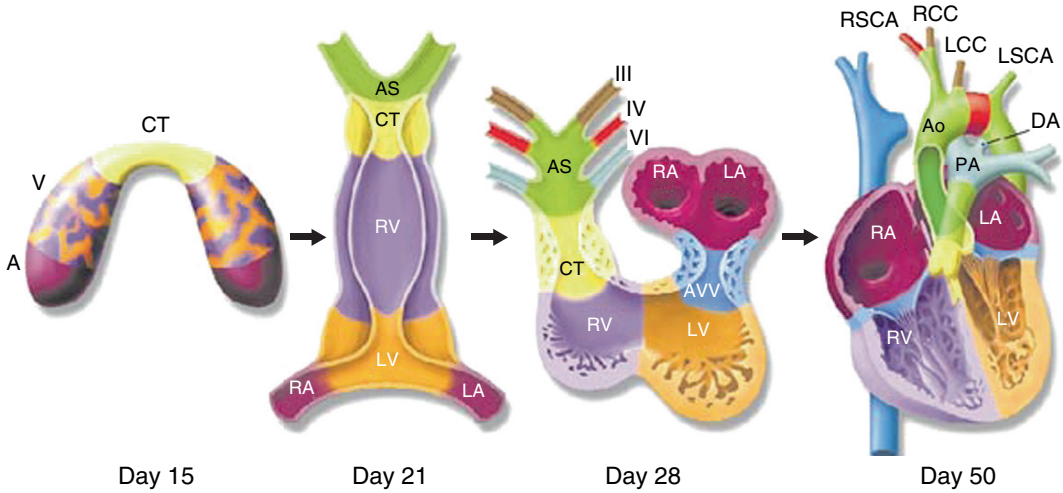

Fig. 13.28 Human heart development. Cardiac development is depicted in four consecutive steps. The heart consists of an inner endocardium, which is the endothelial sheet, and the outer myocardium, which is contractile. The first sign of the developing heart is the formation of a crescent (*left-most panel*), which fuses along the midline to give rise to a tube; this is patterned along the anterior-posterior axis to form the various chambers of the heart. This two-chambered heart is the basic adult form in fish, but in higher vertebrates the tube undergoes looping, a process controlled by the left-right asymmetry of the antero-posterior axis, and further partitioning to form a four-chambered heart. Cells from neural crest origin contribute to the outflow tracts populate the bilaterally symmetrical aortic arch arteries (III, IV, and VI) and aortic sac (AS) that together contribute to specific segments of the mature aortic arch. Mesenchymal cells form the cardiac valves from the conotruncal (CT) and atrioventricular valve (AVV) segments. Corresponding days of human embryonic development are indicated (*A* Atrium, *Ao* Aorta, *DA* Ductus arteriosus, *LA* Left atrium, *LCC* Left common carotid, *LSCA* Left subclavian artery, *LV* Left ventricle, *PA* Pulmonary artery, *RA* Right atrium, *RCC* Right common carotid, *RSCA* Right subclavian artery, *RV* Right ventricle, *V* Ventricle) (Reprinted from [31], by permission from Macmillan Publishers Ltd, *Nature*, copyright 2000)

defects. In the current model, this cilia-directed flow creates an asymmetric accumulation of growth factors such as Nodal and/or Shh that subsequently governs asymmetry. In Kartagener syndrome this is presumably disturbed because the mutations in dynein result in nonfunctional cilia.

The individual segments of the heart can only function if the cardiac valves are properly placed to ensure the unidirectional flow of blood through the heart. Development of the valves starts with the formation of regional swellings, known as cardiac cushions, that form the anlage of the valves. To form the valves a transformation of endocardial cells into mesenchymal cells has to take place, mediated by members of the TGFβ family. Inactivation of Smad6, a transcription factor activated upon TGFβ signaling leads to abnormally thick-ended, gelatinous valves. Transformed cells migrate into the cushions and differentiate into the fibrous tissue of the valves. Atrioventricular canal or endocardial cushion defect represents a developmental abnormality of this process, a congenital heart defect frequently observed in trisomy 21. However, which gene(s) cause this defect is unknown.

Many other genes have been implicated with congenital heart defects. Mutations in TBX5 cause Holt–Oram syndrome, a condition characterized by heart defects, frequently associated with arrhythmia, and limb malformations. TBX5 was shown to be an essential factor for the development of the right ventricle and the outflow tract [3]. In addition, Tbx5 appears to function in the left ventricle and atria by influencing the expression of other transcription factors and proteins that are required for cardiac function. Tbx5 associates with Nkx2.5, another transcription factor shown to be essential for normal heart development. Mutations in NKX2.5 have been identified in families with atrial septal defects and cardiac conduction abnormalities [2]. Sporadic mutations have also been found in individuals with outflow tract alignments defects such as tetralogy of Fallot, or tricuspid valve anomalies. An evolving general theme of congenital heart defects appears to be a considerable variability in the type and severity of cardiac malformations among individuals with mutations in the same gene or even with the same mutation. Mutations in one gene are not predictive for a certain heart defect but rather appear to be responsible for a range of abnormalities, with certain types of defects occurring more often than others.

13.8 Development of the Kidney

The urogenital system, i.e., the kidneys, the gonads, and their respective duct systems, develop from the intermediate mesoderm, the region located between the lateral plate mesoderm (origin of the limbs) and the paraxial mesoderm (origin of the somites). Development of the permanent kidney, the metanephros, is preceded by the formation of a transient structure, the pronephros. The pronephric duct is the first visible sign, a bilateral structure consisting of a single-cell-thick epithelium that extends caudally until it reaches the cloaca. Connected to this tube a linear array of tubules derived from mesenchymal cells adjacent to the primary nephric duct forms. The more caudal tubules contain glomeruli and convoluted proximal tubule-like structures that serve as transient filtration units that degenerate as the development of the permanent kidney takes place [5]. Figure 13.29 shows a summary of kidney development.

The adult kidney or metanephros begins to develop when an outgrowth of the primary nephric duct, termed the ureteric bud, extends into the surrounding metanephric mesenchyme. The ureteric bud branches and first mesenchymal cells begin to aggregate near the tips of the bud. This interaction of mesenchyme with epithelium drives the entire following developmental process. The aggregates first form a polarized renal vesicle, which is still in contact with the bud. Subsequently the so-called comma and S-shaped bodies form and become polarized along a proximo-distal axis as they undergo mesenchyme-to-epithelial conversion. Subsequently the distal end fuses with the ureteric bud to form a single, continuous epithelial tube, which in its distal part will give rise to the proximal tubule, Henle's loop, and the distal tubule. Endothelial cells invade the cleft of the S-shaped body forming the glomerular filtration unit. Interactions between endothelial cells and the mesenchyma-derived glomerular epithelial cells lead to the formation of podocytes and thus the glomerula basement membrane that serves as a filtration barrier. This process of branching, aggregation, mesenchyme-to-epithelial conversion, fusion, and endothelial invasion continues from the inside to the outside in such a way that the oldest nephrons are located closer to the medulla and the youngest ones more peripherally in the nephrogenic zone. In humans this process continues during the

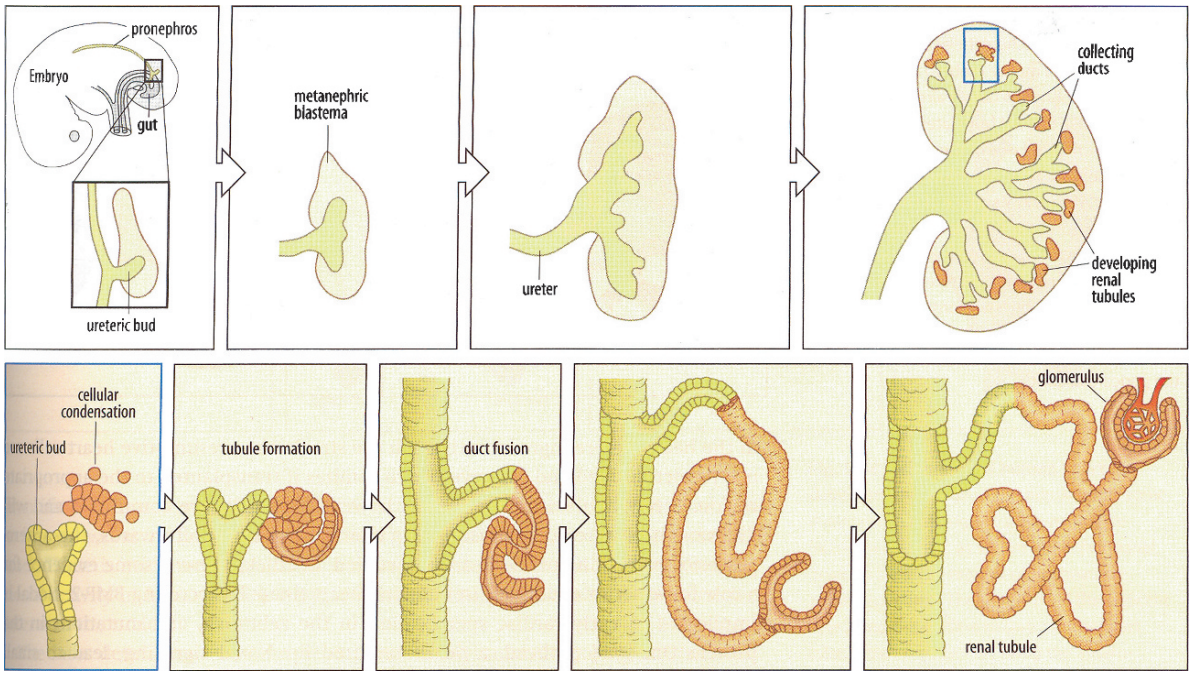

Fig. 13.29 Kidney development. The permanent kidney develops from a loose mass of mesenchyme, the metanephric blastema, which is induced by cells from the ureteric bud. The ureteric bud itself is induced by the mesenchyme to grow and branch. The metanephric blastema eventually develops into the glomerulus and the renal tubule, whereas the ureteric bud will form the collecting ducts that connect the kidney to the ureter. The formation of glomeruli involves several distinct steps, including comma- and S-shaped bodies. The human kidney continues to grow from metanephric blastema until birth. (From [35], p. 377, Fig. 9.46)

entire fetal period until the final size of the kidney is reached.

This complex process in governed by a large number of signaling pathways, including the *Pax*/*Eya*/*Six* genes as well as *Lim1* and *Odd1*. Ureteric outgrowth and branching morphogenesis are controlled by the Ret/Gdnf pathway. In the mouse *Wnt9b* and *Wnt4* genes are critical for aggregation and transformation of metanephrogenic mesenchyme into tubular epithelium. If separated from the utereteric bud mesenchymal cells die. FGF2 and BMP7 are two factors secreted by the ureteric bud that prevent apoptosis and promote aggregation. The competence to respond to uteric bud inducers is thought to be regulated, among other genes, by Wt1, a transcription factor originally found to be mutated in a heritable form of childhood kidney tumor, the Wilms tumor. If this factor is missing, the uninduced cells of the metanephrogenic mesenchyme die and no kidney is formed. Later in development Wt1 is found expressed in podocytes of the glomerular basement membrane. Besides leading to tumors, specific mutations in WT1 can result in Denys–Drash syndrome, a condition characterized by Wilms tumors, nephrotic syndrome (severe proteinuria) leading to end-stage renal failure before the age of 3 years, and pseudohermaphroditism with children having either ambiguous external genitalia or a normal female phenotype with an XY karyotype. The latter phenotype points to an important role of Wt1 in genital development. It is similar to the renal phenotype in that Wt1-deficient mice develop no gonads.

The caudal part of the ureteric bud becomes the ureter, which inserts into the bladder and is a part of the urogenital system that, if nonfunctional, will result in hydronephrosis. The bladder develops out of the cloaca, which is divided by a septum into the rectum und the urogenital sinus. The latter will also give rise to the urethra. *Hox* genes appear to be important for this process. Mutations in HOXA13 result in hand-foot-genital syndrome, a condition characterized by short thumbs/toes and abnormalities of the cloaca, the male and female reproductive tracts and the urethra.

13

13.9 Skeletal Development

The skeleton arises from three distinct sites: the axial skeleton consisting of the vertebrae and ribs originates from the somites, the appendicular skeleton has its origin in mesenchymal cells located in the lateral plate mesoderm, and most of the craniofacial bones are of neural crest origin. Patterning genes determine the number, size, and shape of the future skeleton and guide cells to migrate to the sites of future skeletogenesis, where they aggregate to form the skeletal anlage. The next step is the overt differentiation of these cells into cartilage-forming chondrocytes in endochondral skeletal elements, or into bone forming osteoblasts in areas of membranous bone formation [16, 17]. Figure 13.30 schematically summarizes skeletal development.

In areas of endochondral bone formation, the condensed cells differentiate into chondrocytes that produce cartilage. The transcription factor Sox9 has been shown to be essential for this process. Sox9 is expressed in the early cartilaginous condensations and, at later stages, in growth plate chondrocytes. One of its regulatory targets is *Col2a1*, the gene encoding the major collagen in cartilage. Sox9 binds specifically to sequences within the first intron of this gene. Mutations in SOX9 cause campomelic dysplasia, a lethal chondrodysplasia characterized by bowed long bones (femur and tibia in particular), small scapula, small rib cage, and sex reversal in XY males. Inactivation of Sox9 in mice causes early lethality, but conditional inactivation in the limbs shows that Sox9 is essential for the differentiation of precursor cells into chondrocytes.

The TGFβ/BMP/GDF5 pathway plays an important role in the regulation of condensation and differentiation of precursor cells into chondrocytes. Signaling of the Tgfβ superfamily members requires the binding of the ligand to cell surface receptors consisting of two types of transmembrane serine/threonine kinase receptors classified as type I and type II. The type II receptor transphosphorylates and thus activates the type I receptor. The intracellular substrates of the activated type I receptors are the Smads. Smads 1, 5, and 8 are phosphorylated and then translocated to the nucleus, where

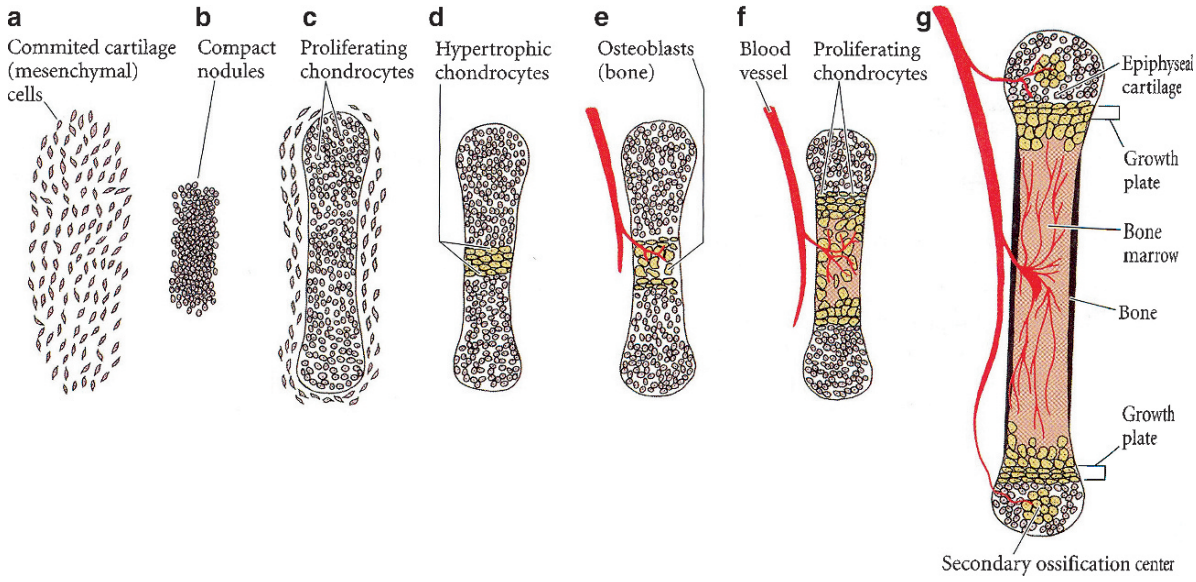

Fig. 13.30 Skeletal development**.** The first sign of skeletogenesis is the condensation of precursor cells at the site of future bone formation. The condensed cells differentiate into cartilage to form the cartilaginous anlage of the future bone or directly into bone at sites of desmal ossification. Chondrocytes in the center of the cartilaginous anlage begin to hypertrophy, and their matrix begins to mineralize. At this stage the first cortical bone begins to form as a thin layer of osteoid around the shaft of the anlage. In addition, vessels invade the cartilage, introducing monocytic progenitor cells that differentiate into osteoclasts that remove cartilage and bone. A growth plate forms at each end of the bone, in which most of the growth is generated until adulthood. Secondary ossification centers develop in the cartilage heads of the bone. (From [10], p. 456, Fig. 14.14)

they participate in the transcriptional regulation of the expression of genes involved in cartilage and bone formation [22]. BMP signaling is controlled by the binding of BMPs to inhibitors in the extracellular space. One potent inhibitor is Noggin, a gene originally identified for its role to induce head formation in *Xenopus*. Activation of BMP signaling either by overexpression, activation of the receptor, or inhibition of the inhibitor results in grossly enlarged cartilaginous anlagen. The likely mechanism for this effect is the recruitment of mesenchymal precursor cells to the cartilage condensations and to the perichondria, which contribute cells to the anlage by appositional growth. Mutations in GDF5, another member of the BMP/TGFβ family or its receptor BMPR1B result in various limb phenotypes, mainly characterized by shortening of the digits (brachydactyly). Mutations in the inhibitor NOGGIN or activating mutations in GDF5 cause joint fusions (symphalangism, synostosis syndrome), indicating that the tight regulation of BMP signaling is also essential for joint formation [29].

In areas of membranous bone formation, the condensed cells differentiate into osteoblasts which produce bone matrix. Genetic experiments in mice have demonstrated that Cbfa1/Runx2 is essential for this process. Runx2 is a member of a small family of transcription factors that are homologous to the *Drosophila* runt gene. In Runx2 null mice no endochondral or membranous bone is formed owing to an arrest in the early steps of osteoblast differentiation (Fig. 13.31). In contrast, the cartilaginous template is relatively normal. Mutations in RUNX2 cause cleidocranial dysplasia, a skeletal dysplasia characterized by patent fontanelles, aplastic/hypoplastic clavicles, supernumerary teeth, and short stature [24].

In contrast to the bones of the skull, which are formed by a direct transformation of mesenchymal cells into osteoblasts, the major part of the skeleton is formed by endochondral ossification in which a cartilaginous template is formed first, which is subsequently replaced by bone. Central to this process is the formation of a growth plate, a highly organized structure that generates the entire longitudinal growth. Growth plate chondrocytes are invariably arranged in three layers: (1) reserve chondrocytes, (2) proliferating chondrocytes, and (3) hypertrophic chondrocytes. A very complex interplay of different signaling pathways regulates the rate of proliferation and the conversion of proliferating chondrocytes into hypertrophic chondrocytes.

Fig. 13.31 (**a, b**) Role of Runx2 in bone development. Inactivation of Runx2 transcription factor results in complete loss of bone. Mouse skeleton with cartilage stained blue and bone stained red. Note *red* and *blue* staining in control (**a**) but no red staining in Runx2−/− mice (**b**)

During the process of chondrocyte differentiation, the matrix changes dramatically through the production of other components such as collagen type 10, the expression of metalloproteinases, and the calcification of matrix. At the same time, blood vessels begin to penetrate the calcified cartilage, bringing in osteoclasts that remove cartilage and osteoblasts, which build new bone. With further growth, the central and primary centers of ossification expand towards the ends of the bones and secondary centers of ossification form within the cartilage remnants. The growth plate, now localized between the epiphysis (secondary center of ossification) and the metaphysis (distal end of former primary ossification center) generates all longitudinal growth until the end of puberty when primary and secondary ossification centers fuse. At this point, the cartilage of the joints is the only cartilage that remains of the former anlage (Fig. 13.32).

Two major signaling pathways that control proliferation and differentiation of chondrocytes have been identified, the Indian hedgehog (*Ihh*)/parathyroid

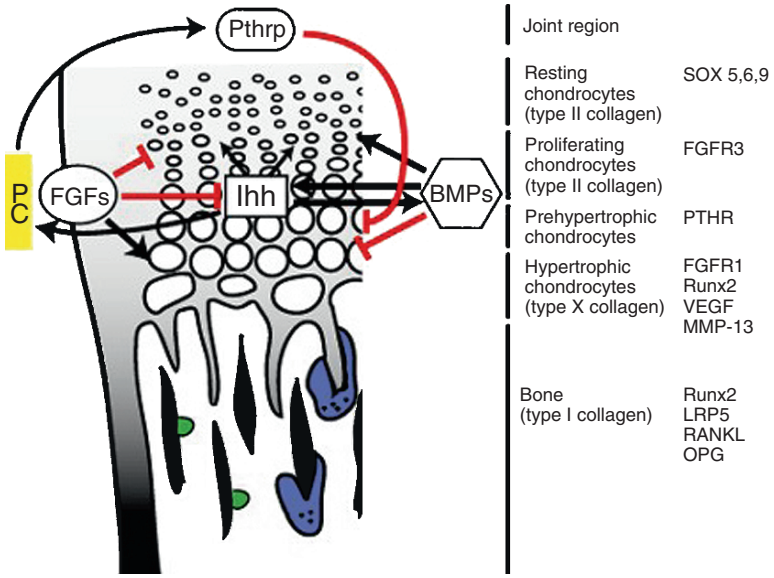

Fig. 13.32 Schematic of growth plate and its regulation. Beginning from the joint region, chondrocytes are arranged in four layers, representing their stages of differentiation: resting, proliferating, prehypertrophic, and hypertrophic chondrocytes. The later are replaced by bone by the joint action of osteoblasts (*green*) and multinuclear osteoclasts (*blue*). Genes that are characteristically expressed within each layer are given on the *right* side. A complex signaling network regulates proliferation and differentiation of chondrocytes. Prehypertrophic chondrocytes express *Ihh*, which regulates PTHrp, which is produced by the joint region, via the perichondrium. PTHrp, in turn, inhibits differentiation of proliferating chondrocytes. FGFs inhibit *Ihh* and chondrocyte proliferation and stimulate differentiation of chondrocytes. BMPs act antagonistically to the FGFs. *Gray shading* symbolizes the degree of calcification of the extracellular matrix

hormone related peptide (PTHrP) pathway and the FGF pathway. Indian hedgehog (*Ihh*), the second mammalian hedgehog ortholog, plays a central role in the regulation of chondrocyte proliferation and hypertrophy. Through a yet unknown cascade of events *Ihh* indirectly regulates the expression of PTHrP. *Ihh*, and PTHrP form a feedback loop whereby *Ihh* up-regulates the synthesis of PTHrP, thereby indirectly slowing down the process of chondrocyte hypertrophy. Mutations in the PTHrP pathway cause pseudohypoparathyroidism (PHP) and pseudopseudoparathyroidism (PPHP). In PHP abnormalities in calcium and phosphate metabolism (hypocalcemia, elevated PTH levels) are accompanied by obesity, mental retardation, short stature, subcutaneous calcifications, and short digits (Albright hereditary osteodystrophy), whereas PPHP patients have osteodystrophy with normal calcium and PTH levels.

FGF signaling plays a major role in the regulation of chondrocyte proliferation and differentiation. More than 20 different FGFs are currently known, and four distinct FGF receptors (FGFR) have been described that bind and are activated by members of the FGF family. FGFs are potent mitogens for chondrocytes as well as for osteoblasts and stimulate bone formation in vitro and in vivo. Inactivation of Fgfr3 in the mouse results in overgrowth of the long bones, whereas expression of an activating mutation results in dwarfism, indicating that Fgfr3 functions as a negative regulator of chondrocyte proliferation and/or hypertrophy. Mutations in FGFR3 cause the most common form of skeletal dysplasia, achondroplasia. Affected individuals are characterized by disproportionate short stature (130 cm adult height). Further activating mutations in FGFR3 cause hypochondroplasia, a less severe variant, and thanatophoric dysplasia, the most severe and lethal form of this dysplasia group.

Cartilage is characterized by a unique extracellular matrix that accounts for around 90% of the tissue volume. The main component is fibrous collagen, which confers tensile strength. The growth plate cartilage contains type II, IX, X, and XI collagen. In the resting and proliferating cartilage type II collagen predominates, which can form fibers together with type IX and type XI. Type X collagen, in contrast, is specific for hypertrophic cartilage. Proteoglycans, and especially

aggrecan, are highly abundant. They are giant molecules, which have a gel-like consistency when dissolved in water. Consisting of a core protein to which different kinds of glycosaminoglycans (GAGs) are attached, the proteoglycans are highly sulfated and thus negatively charged. This allows them to bind large amounts of cations and water molecules and to be mutually repellent, which is thought to contribute to the elasticity of the cartilage. Glycoproteins are a third group of matrix components comprising for example perlecan, fibronectin, tenascin, and cartilage oligomeric matrix protein (COMP). Mutations in any of these cartilaginous matrix components result in skeletal dysplasias with growth deficiencies.

In bone, type 1 collagen is the most abundant protein. In contrast to cartilage, bone matrix contains few proteoglycans, but instead consists largely (two thirds) of hydroxyapatite, to ensure that it is rigid. Mutations in either of the two genes that encode for the two chains that contribute to collagen type I (COL1A1 and COL1A2) result in osteogenesis imperfecta, a group of diseases primarily characterized by brittleness of bones and recurrent fractures (Fig. 13.33).

Homeostasis of bone mass and remodeling during skeletal growth are accomplished by an antagonistic action of bone producing osteoblasts and bone resorbing osteoclasts. Osteoclasts originate from mononuclear hematopoietic precursor. When osteoclasts attach to bone they create the so-called ruffled membrane, which contains high levels of V-type H^+-ATPases that pump protons into the tightly sealed resorption lacuna between osteoclast and bone surface. Strong extracellular acidification is crucial for bone resorption, as low pH is needed to dissolve the hydroxyapatite of the bone tissue and to degrade the organic components of the bone matrix, above all type I collagen, by acidic proteases such as cathepsin k. Dysfunction of osteoclasts results in an abnormal accumulation of bone also called osteopetrosis. The recessive forms are life-threatening conditions caused by loss of the bone marrow cavity. They can be caused by defects in the osteoclast acidification machinery, either affecting the osteoclast proton pump TIGR, the chloride transporter CLC-7 [18], or carboanhydrase type II, a protein responsible for synthesizing the protons transported by the H^+-ATPase. Mutations in cathepsin k, which is indispensable for bone resorption since it is the only protease able to cleave the intact collagen triple-helix, result in the sclerosing disorder pycnodysostosis.

13.10 Abnormal Development: Definitions and Mechanisms

The complexities of embryonal development are reflected by the vast number of phenotypes produced by abnormal development. Anomalies that are of prenatal

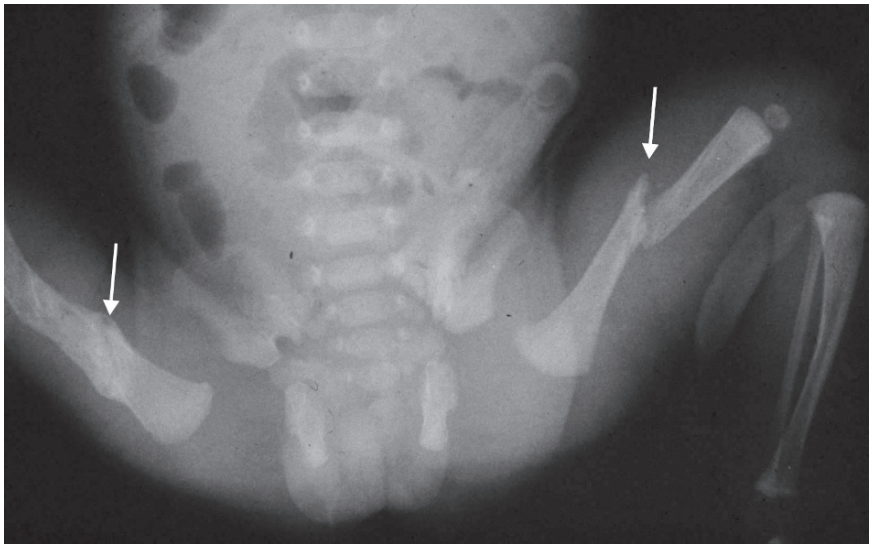

Fig. 13.33 Newborn with type II osteogenesis imperfecta**.** Note fresh fracture of left femur and old fracture of right femur with callus formation (*arrows*)

onset are generally subsumed under the heading congenital. Such defects are either visible at birth or can be diagnosed at a later time point when they manifest as disease (e.g., deafness, anomalies of teeth). 'Congenital' does not necessarily mean genetic or inherited, because congenital defects can also be caused by maternal disease (e.g., diabetes), intraterine infections (e.g., rubella), or mechanical forces (e.g., uterine constraint caused by oligohydramnion).

Congenital anomalies contribute to a great extent to neonatal morbidity and mortality. It has been estimated that, depending on the methodology of assessment, 4–8% of all newborns are born with a medically relevant anomaly. Thus, approx. 1 in 20 newborns has a recognizable and medically relevant anomaly. This proportion is higher among children who die during the neonatal phase (20%) and is much higher in miscarried babies. In Western countries congenital anomalies are the most frequent cause of neonatal mortality.

Abnormalities of development will have a different outcome depending on the cause, time point, and magnitude of the insult. For example, defects that affect the embryo during the preimplantation phase usually result in either complete restoration or loss of the embryo, whereas deficiencies that occur later can result in either death of the embryo or in birth defects. For the clinician a careful clinical analysis may reveal important information that is of high relevance not only for understanding the condition, but also for successful counseling. As illustrated in Fig. 13.34, all developmental anomalies can be categorized into four subgroups.

13.11 Malformations

Primary malformations are caused by an intrinsic defect of the embryo, i.e., they are genetic or due to an interaction of the embryo's genome with its environment. They can be inherited or may develop as a result of a de novo mutation that has occurred in the oocyte or the sperm, or during early embryogenesis. Malformations are due to inactivation or dysregulation of important developmental genes during the postimplantation phase. These genes regulate early pattering processes and/or organogenesis. Because regulation of early development is their most important role, the developmental defect happens very early and the

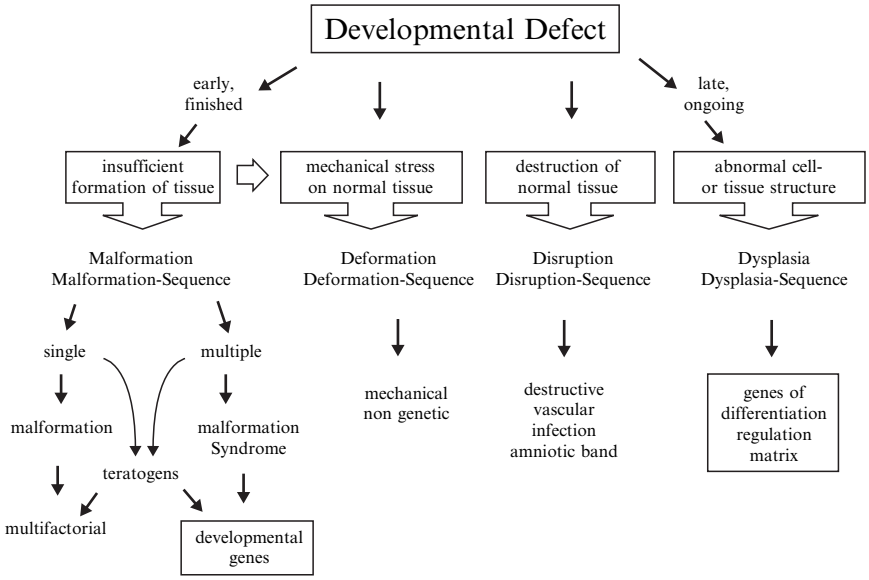

Fig. 13.34 Types of structural defects. Congenital defects can be subdivided in four major groups, malformations, deformations, disruptions, and dysplasias. As shown, their etiology can be genetic, multifactorial, and non-genetic. Each type may induce secondary changes, also called sequence. (From [20], p. 282, Fig. 31.2)

pathogenesis of the condition is thus completed at the time point when it is observed.

The majority of malformations are likely to be caused by a combination of environmental and polygenic factors. Abnormalities in the maternal metabolism, such as diabetes mellitus, are such an environmental factor which, together with yet unknown susceptibility genes, may result in severe malformations, such as caudal regression. A smaller number of birth defects is due to chromosomal aberrations or is caused by single gene defects.

Defects caused by polygenic factors are most commonly "single site defects," i.e., they affect one site or organ of the body while leaving others intact. They occur at relatively high frequency and are either present or absent, i.e., they show little overlap with minor defects. Such malformations include cleft lip and palate, isolated cleft palate, neural tube defects, club foot, congenital heart defects, and pyloric stenosis to mention the most frequent ones. They tend to cluster in families but do not conform to Mendel's laws of gene transmission. Risk calculation has determined that: (1) first-degree relatives have a risk approximating the square root of the population risk; (2) second-degree relatives have a sharply lower risk than first-degree relatives; (3) the higher the number of affected individuals in one family, the higher the risk; (4) consanguinity increases the risk; and (5) the more severe the malformation, the higher the risk. For some defects the influence of environmental factors is larger than for others. For example, neural tube defects can be prevented to a large extent if folic acid is supplemented during early pregnancy. In fact, the folate-neural-tube defect relation represents the only instance in which a congenital malformation can be prevented by changing the environment. In contrast, the frequency of concordance and discordance in monozygotic and dizygotic twins argues against both a single-gene etiology and a major environmental influence in most other conditions.

Cleft lip with or without cleft palate is one of the most common birth defects in the world, with an average prevalence of 1/700. Transforming growth factor (TGFA) a is a growth factor expressed during palatogenesis in the mouse, but mice with inactivation of TGFA have abnormal skin, hair, and eyes, but no clefts. TGFA was found to be associated with human clefts in a first study in 1989 and has been confirmed in several studies since. Taken together, these studies show that TGFA is probably a modifier of clefting in humans, which is consistent with an oligogenic model for clefting in humans. Several causative genes for inherited syndromic forms of cleft lip/cleft palate have been identified and some studies show that they may also contribute to the occurrence of isolated forms.

In contrast to the above-mentioned polygenic conditions, chromosomal imbalances and single-gene disorders are usually associated with a combination of anomalies, hence their name "syndrome" (Greek, meaning "convergence"). For the clinician this means that the search for other associated anomalies is pivotal, since it helps to distinguish single-gene defects from polygenic conditions. As only the combination of symptoms defines a syndrome, a clinical diagnosis cannot be made on the basis of a single defect. In that respect the detection of minor defects may be as helpful as that of major anomalies. The pattern of anomalies defines the condition and, in addition, the functional role of the gene affected. Chromosomal aberrations, including microdeletions, show their "own" pattern, which is usually less defined and broader (e.g., a combination of specific patterning defects with mental retardation) than that of single gene defects because multiple genes are involved that may act in a additive or even epistatic way.

Malformations affecting single sites may result in consecutive changes of other sites, giving rise to a malformation sequence. For example, innervation problems of the tongue resulting in a reduced pressure on the lower jaw result in reduced growth of the mandible, also called micrognathia. The latter is a consequence of the initial defect and thus secondary. Arthrogryposis is a congenital contracture of the fingers with partial joint fusions. In most cases this is not due to a primary defect in joint formation, but to a secondary effect caused by reduced fetal movement either because of reduced muscle mass or reduced muscle innervation. Both examples illustrate that the most obvious abnormality is not necessarily the most informative. Most primary defects lead to secondary effects, and sometimes it is difficult to separate cause from effect.

13.12 Disruptions

Disruptions define destructive problems acting on a normal fetus. The fetus has the ability to develop normally but fails to do so. Such disruptions may be of

vascular, infectious, or teratogenic origin. They usually affect several different tissues in a particular anatomic region without adhering to regions defined by developmental processes. For example, an amniotic band can cut through a certain part of an embryo, destroying muscle, skin, bone, and other tissues that are not embryologically related. Probably secondary to amnion rupture, small strands of amnion can encircle developing structures (most frequently the limbs), leading to annular constrictions, edema, disruptive necrosis, amputation, or syndactyly. At birth aberrant strands can be noted and/or remnants of the rolled-up amnion are present at the placental base. In the milder cases this leads to asymmetric amputation-like phenotypes of the digits. In the most severe cases the entire limb may be lost and secondary deformational defects consequent on decreased fetal movement may occur. Other disruptions can be caused by intrauterine infections: rubella, for example, leads to the destruction of certain organ systems (in this case the inner ear) that would have developed in a regular way if the insult had not occurred. Teratogenic substances such as thalidomide or valproate may cause disruptions because they interfere with normal signaling and/or cell proliferation

13.13 Deformations

In contrast to malformations, which are caused by a primary and intrinsic problem in morphogenesis, and disruptions, which represent the breakdown of a previously normal tissue, deformations are abnormalities caused by mechanical forces. They usually occur during the late phase of pregnancy, but can nevertheless have a pronounced influence on fetal development. Deformations may be intrinsic, i.e., due to abnormal development of the fetus itself, or extrinsic, produced by constraint in utero of an otherwise normal fetus. Such extrinsic forces may produce a single localized deformation, such as a deformed foot, or they may cause a deformation sequence. The potter sequence is an example for the complex effects of mechanical forces. In cases of serious oligohydramnion caused by amniotic fluid leakage or due to reduced production of fluid (aplasia of kidneys, urethral valves, etc.) thoracic growth is restrained and the full growth and maturation of the lungs is thwarted, making them incapable of aerobic expansion and oxygen exchange. The nose is flattened and the limbs are in an aberrant position, often with stiff joints owing to insufficient movement. Breech presentation is another important cause of deformation. Owing to the abnormal position in utero, the head is elongated, approaching a scaphocephalic form, and there are redundant skin folds in the posterior neck, presumably due to the constant retroflexion of the head. The legs are usually hyperflexed in front of the fetus, and any gradation of hip dislocation may occur. Most of the deformations have an excellent prognosis once the fetus is released from the constraining environment. However, some need treatment to release contractures, while others are difficult to treat if the deformation interferes with normal organ development.

13.14 Dysplasias

Dysplasias are caused by gene defects that affect the formation and growth of tissues. In contrast to malformations that are due to abnormal events during early embryonic patterning, dysplasias affect the embryo at a later time point, when the patterning phase is completed. Accordingly, the gene defects that cause dysplasias frequently continue to be active after birth. This offers potential treatment options that are hard to envision for malformations. On the other hand, owing to the ongoing gene activity, some dysplasias carry the potential risk for malignant transformation. Mutations in the cartilage-specific COL2A1 gene cause different forms of skeletal dysplasias ranging from lethal types (achondrogenesis), severe dwarfism (SED congenita), to normal stature with premature osteoarthrosis and myopia. Even the most severe cases have normal skeletal patterning, i.e., they have a normal number of fingers, toes, vertebrae, etc., but all bones are very short owing to a defect in tissue (cartilage) formation. Likewise, mutations in one of the collagen type 1 chains (COL1A1, COL1A2) cause brittle bone disease or osteogenesis imperfecta. Here the affected tissue is not cartilage but bone, because COL1A1 and COL1A2 are predominantly expressed in bone and are essential for the mineralization of bone and thus for its strength.

13.15 Terminology of Congenital Defects

Congenital defects come in a bewildering diversity. Further complicating the situation is the extreme variability in some conditions combined with reduced penetrance. Over the past clinical geneticists set out to order these conditions by giving names to diseases that looked similar. Fine discrimination of the phenotype is necessary to distinguish similar entities. For example, achondroplasia was frequently misdiagnosed among individuals who have small stature and chondrodysplasia that only superficially resemble true achondroplasia. Only the clear delineation of signs and symptoms made definite diagnosis on clinical grounds possible. This phenotypic approach proved to be very successful, since most clinically defined conditions were subsequently identified as having similar or identical gene defects. Certain defects that occurred together were defined as a "syndrome," implying that all patients with this condition are likely to have the same gene defect. The identification of mutations in these patients has shown that the theory holds true, at least in principle. However, similar phenotypes may also be caused by different gene defects, a phenomenon known as genetic heterogeneity. It turns out that gene defects that result in similar phenotypes are likely to be linked within a common molecular pathway. The disruption of such a pathway results in a certain phenotype regardless of where it takes place. Noonan syndrome, for example, is a relatively common condition characterized by small stature, characteristic facies, and heart defects. Mutations in KRAS, a proto-oncogene well known from its mutations in tumors, have been shown to be responsible for Noonan syndrome. Mutations in other components of the Ras pathway including PTPN11, SOS1, and HRAS were shown to cause Noonan or Noonan-like phenotypes illustrating how defects in one pathway may lead to overlapping phenotypes [9]. Conditions that are caused by gene mutations within one molecular pathway and that are thus associated with similar phenotypes have also been called "molecular disease families" [23]. The ongoing identification of gene defects associated with specific phenotypes will eventually enable us to link phenotype and genotype and to better understand differences and similarities in human birth defects.

References

1. Ashe HL, Briscoe J (2006) The interpretation of morphogen gradients. Development 133:385–394
2. Benson DW, Silberbach GM, Kavanaugh-McHugh A, Cottrill C, Zhang Y, Riggs S, Smalls O, Johnson MC, Watson MS, Seidman JG, Seidman CE, Plowden J, Kugler JD (1999) Mutations in the cardiac transcription factor NKX2.5 affect diverse cardiac developmental pathways. J Clin Invest 104:1567–1573
3. Bruneau BG, Nemer G, Schmitt JP, Charron F, Robitaille L, Caron S, Conner DA, Gessler M, Nemer M, Seidman CE, Seidman JG (2001) A murine model of Holt–Oram syndrome defines roles of the T-box transcription factor Tbx5 in cardiogenesis and disease. Cell 106:709–721
4. Cordero D, Marcucio R, Hu D, Gaffield W, Tapadia M, Helms JA (2004) Temporal perturbations in sonic hedgehog signaling elicit the spectrum of holoprosencephaly phenotypes. J Clin Invest 114:485–494
5. Dressler GR (2006) The cellular basis of kidney development. Annu Rev Cell Dev Biol 22:509–529
6. Driever W, Nusslein-Volhard C (1988) A gradient of bicoid protein in Drosophila embryos. Cell 54:83–93
7. Driever W, Nusslein-Volhard C (1989) The bicoid protein is a positive regulator of hunchback transcription in the early Drosophila embryo. Nature 337:138–143
8. Evans PD, Gilbert SL, Mekel-Bobrov N, Vallender EJ, Anderson JR, Vaez-Azizi LM, Tishkoff SA, Hudson RR, Lahn BT (2005) Microcephalin, a gene regulating brain size, continues to evolve adaptively in humans. Science 309:1717–1720
9. Gelb BD, Tartaglia M (2006) Noonan syndrome and related disorders: dysregulated RAS-mitogen activated protein kinase signal transduction. Hum Mol Genet 15(Spec No 2):R220–R226
10. Gilbert SF (2006) Developmental biology, 8th edn. Oxford University Press, Oxford
11. Gridley T (2006) The long and short of it: somite formation in mice. Dev Dyn 235:2330–2336
12. Guerrini R, Marini C (2006) Genetic malformations of cortical development. Exp Brain Res 173:322–333
13. Haltiwanger RS (2002) Regulation of signal transduction pathways in development by glycosylation. Curr Opin Struct Biol 12:593–598
14. Ingham PW, McMahon AP (2001) Hedgehog signaling in animal development: paradigms and principles. Genes Dev 15:3059–3087
15. Jacob J, Briscoe J (2003) Gli proteins and the control of spinal-cord patterning. EMBO Rep 4:761–765
16. Karsenty G (2003) The complexities of skeletal biology. Nature 423:316–318
17. Kornak U, Mundlos S (2003) Genetic disorders of the skeleton: a developmental approach. Am J Hum Genet 73:447–474
18. Kornak U, Kasper D, Bosl MR, Kaiser E, Schweizer M, Schulz A, Friedrich W, Delling G, Jentsch TJ (2001) Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. Cell 104:205–215
19. Krumlauf R (1992) Evolution of the vertebrate Hox homeobox genes. Bioessays 14:245–252

20. Lentze M, Schaub J, Schulte FJ, Spranger J (2007) Pädiatrie: grundlagen und praxis, 3rd edn. Springer, Heidelberg
21. Maeda RK, Karch F (2006) The ABC of the BX-C: the bithorax complex explained. Development 133:1413–1422
22. Massague J, Chen YG (2000) Controlling TGF-beta signaling. Genes Dev 14:627–644
23. Mundlos S (2009) The brachydactylies - a molecular disease family. Clin Genet 76:123–136
24. Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, Lindhout D, Cole WG, Henn W, Knoll JH, Owen MJ, Mertelsmann R, Zabel BU, Olsen BR (1997) Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. Cell 89:773–779
25. Ramsdell AF (2005) Left-right asymmetry and congenital cardiac defects: getting to the heart of the matter in vertebrate left-right axis determination. Dev Biol 288:1–20
26. Reiner O, Coquelle FM (2005) Missense mutations resulting in type 1 lissencephaly. Cell Mol Life Sci 62:425–434
27. Roessler E, Muenke M (1998) Holoprosencephaly: a paradigm for the complex genetics of brain development. J Inherit Metab Dis 21:481–497
28. Schwabe GC, Mundlos S (2004) Genetics of congenital hand anomalies. Handchir Mikrochir Plast Chir 36:85–97
29. Seemann P, Schwappacher R, Kjaer KW, Krakow D, Lehmann K, Dawson K, Stricker S, Pohl J, Ploger F, Staub E, Nickel J, Sebald W, Knaus P, Mundlos S (2005) Activating and deactivating mutations in the receptor interaction site of GDF5 cause symphalangism or brachydactyly type A2. J Clin Invest 115:2373–2381
30. Smith JC (1994) Hedgehog, the floor plate, and the zone of polarizing activity. Cell 76:193–196
31. Srivastava D, Olson EN (2000) A genetic blueprint for cardiac development. Nature 407:221–226
32. Tickle C (2002) Molecular basis of vertebrate limb patterning. Am J Med Genet 112:250–255
33. Vincent J-P, Briscoe J (2001) Morphogens. Curr Biol 11(21):R851–854
34. Wiedemann HR, Kunze J, Dibbern H (1989) Atlas der klinischen Syndrome (Atlas of clinical syndromes) fuer Klinik und Praxis, 3rd edn. Schattauer, Stuttgart
35. Wolpert L Principles of development, 3rd edn. Oxford University Press, Oxford