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10.

Development of the Craniofacial Complex

Rena N. D'Souza, L-Bruno Ruest, Robert J. Hinton, and Kathy K. H. Svoboda

10.1 Introduction

The craniofacial complex comprises the head, face, and oral cavity and is the most distinguishing of all the structures in the human body, imparting unique identities to individuals. Structures of the craniofacial complex, such as the mandible, palate, temporomandibular joint (TMJ), and dentition, each offer valuable paradigms for studying development, structure, and functions. This chapter will provide a background for the succeeding chapter that will compare, in detail, the differences in bone and tooth development. The chapter's goals are to review the classical and current knowledge of branchial arch development, mandibular and temporomandibular joint (TMJ) formation, palatogenesis, and tooth development. Information on defects or disorders that arise from perturbations in genes, their protein products, and relevant signaling pathways is integrated into each section. The overarching goal is to emphasize how the knowledge of fundamental developmental processes can be translated to regenerative approaches targeted at restoring the integrity and function of craniofacial tissues.

10.2 Development of the Craniofacial Skeleton

Ossification of the skeleton of the head utilizes both endochondral and intramembranous processes (Fig. 10.1). The neurocranium that comprises the cranial base that underlies the brain is formed by endochondral ossification of cartilages that originate from the mesoderm (chondrocranium). In contrast, the neurocranium creating the cranial vault develops from the cells from the paraxial mesoderm or neural crest that undergo intramembranous ossification (Fig. 10.1) [31, 35, 76, 150]. The cranial sutures between the mesodermal bones involve cells originating from the neural crest. The skeleton of the face, i.e., the viscerocranium, develops from the neural crest cells that undergo both intramembranous and endochondral ossification (Fig. 10.1). After delaminating from the neural crest, these cells undergo an epithelial-to-mesenchymal transition and eventually migrate anterior to the developing brain and into the pharyngeal arches, which are structures located on the ventral aspect of the embryos. Each arch produces a specific cartilage. Two bilateral cartilaginous rods known as

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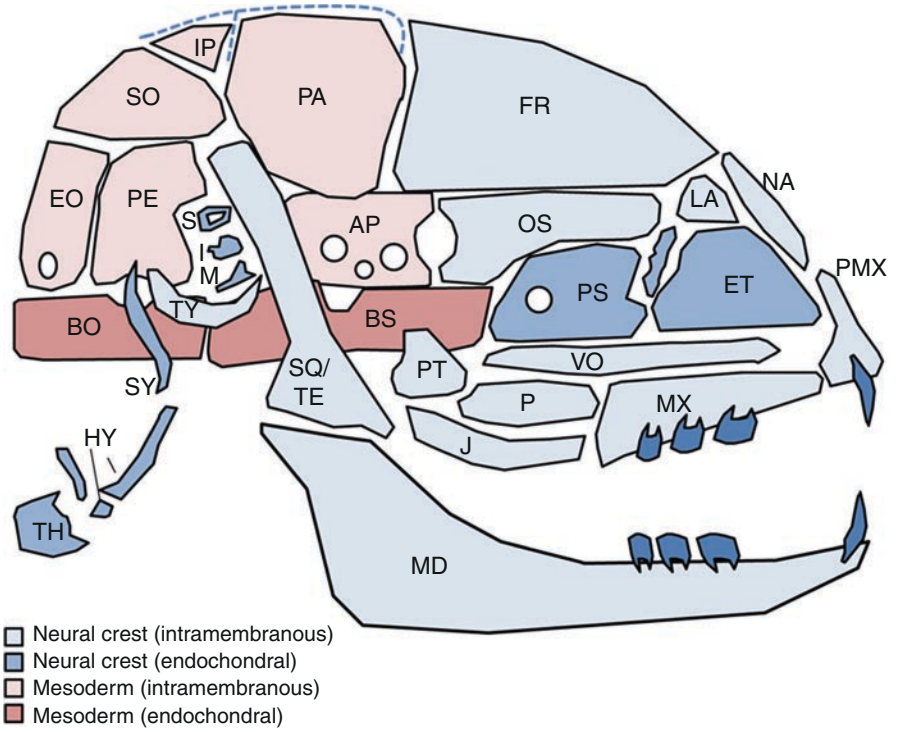

Figure 10.1. Mouse skull showing the contributions of neural crest and mesodermal mesenchyme to the cranial skeleton. Darker colors indicate skeletal structures that undergo endochondral ossification, and lighter colors indicate structures that are formed by intramembranous ossification. As teeth do not form by endochondral or intramembranous ossification, they are shown in another hue of blue to indicate neural crest cell contributions during their formation. *AP* alisphenoid; *BO* basoccipital; *BS* basisphenoid; *EO* exoccipital; *ET* ethmoid; *FR* frontal; *HY* hyoid components; *I* incus; *IP* interparietal; *J* jugal (zygoma); *LA* lacrimal; *M* malleus; *MD* mandible; *MX* maxilla, *NA* nasal; *OS* orbitosphenoid; *P* palatine; *PA* parietal; *PE* petrosal (including mastoid process); *PMX* premaxilla; *PS* presphenoid; *PT* pterygoid; *S* stapes; *SO* supraoccipital; *SQ/TE* squamosal/temporal; *SY* styloid process; *TH* thyroid; *TY* tympanic ring; and *VO* vomer. (Adapted from Noden and Trainor [150], with permission from Wiley-Blackwell, and from Chai and Maxson [35], copyright 2006, reprinted with permission from Wiley).

"Meckel's cartilages" are associated with the first arch and give rise to the lower jaw (mandible) and the malleus and incus. The second arch produces a part of the body and the lesser horns of the hyoid (Reitchert's cartilage), styloid process, and stapes; the third arch gives rise to the body and greater horns of the hyoid. The thyroid cartilage and the cricoid cartilage are created from the fourth and the sixth arches, respectively. The first pharyngeal arch has typically been subdivided into the mandibular and maxillary (upper jaw) prominences. Mapping data indicate that the maxillary prominences contribute to the formation of structures that are associated with the lower jaw [34, 116, 172], with the maxilla, palatine, and jugal (zygomatic arch) originating from the neural crest cells that migrate toward the postoptic region above the maxillo-mandibular cleft.

The development of the facial skeleton above the mandible involves the orderly and integrated growth and movement of different prominences that eventually fuse and expand to form the face [31, 35, 76, 150]. Fusion failure results in orofacial clefts. The five different facial prominences are the frontonasal, medionasal (2), nasolateral (2), maxillary (2), and mandibular (2). The mandibular prominences, constituting the first pharyngeal arch, fuse at the midline days before the other prominences fuse. The development of the first pharyngeal arch is discussed subsequently. The frontonasal prominence grows downward and forms the forehead, middle of the nose, philtrum of the upper lip, premaxilla, and primary palate. The medial and lateral nasal prominences develop on each side of the nasal pit, which becomes the nostrils.

These prominences eventually migrate medially and fuse with the frontonasal prominence to form the nose. The maxillary prominences also move forward toward the midline and fuse with the frontonasal prominence to complete the upper lip and, with the lateronasal prominences, form the nasolacrimal grooves. The maxillary prominences form the sides of the face and the associated bones of the zygomatic arches, maxilla, and secondary palate.

During the process of bone formation, the endochondral and intramembranous bones use the same mechanisms of osteoblast differentiation, similar to the axial and appendicular skeletons [101, 110]. As *Runx2*-null mice [109, 155] are born without endochondral and intramembranous bones, it is evident that the transcriptional factor, Runx2, is essential for bone differentiation. The activation of *Sox9* is also required for the formation of the osteo-chondro-progenitor that will differentiate into the chondrocytes [78, 125]. The differentiation of the chondrocytes into hypertrophic chondrocytes and the subsequent differentiation steps, including ossification, are mediated by *Runx2*; Sox9 activity is not essential for intramembranous bone formation.

10.3 Development of the Mandible

The mandible is a unique mammalian structure that allows mastication during feeding. Its development involves cells that, originating from the neural crest, populate the first pharyngeal arch. These cells are influenced by signals from the ectoderm, core paraxial mesoderm, and pharyngeal pouch endoderm that induce the endochondral differentiation of the neural crest cells to create the two bilateral cartilaginous rods known as "Meckel's cartilages" and differentiate into the osteoblasts forming the intramembranous bone.

Mandibular development begins with the condensation of the neural crest mesenchymal cells that form the mandibular blastema and continues as a subpopulation of these cells differentiates into chondrocytes that then form Meckel's cartilage. The two rods of the proximal Meckel's cartilage differentiate into the malleus. The distal ends of the Meckel's cartilage project inward toward the embryo midline, where they meet at the site of the eventual symphysis of the lower jaw.

Meckel's cartilage serves as a scaffold for the ensuing ossification [168, 169, 220]. Unlike long bones that undergo endochondral ossification, the mandible ossifies mostly through an intramembranous process. During embryogenesis, the ossification of the mandible starts on the external side along the first distal third of the Meckel's cartilage and proceeds rapidly toward the oral side [168, 169]. Ossification then ensues along the cartilage, proximally and distally. The ossification of the condylar and coronoid processes represents a continuous addition of intramembranous bone and occurs independently of the scaffold provided by the Meckel's cartilage.

Interestingly, part of the neural crest-derived mesenchyme does not differentiate into bone, but provides the alveolar spacing essential for tooth development. Skeletal muscles develop along the ossification front, initiating the jaw-opening reflex [106]. This initial jaw opening articulates with the developing incus via the Meckel's and malleus cartilages and is reminiscent of the articulation between the articular of the lower jaw and the quadrate (ancestral incus) seen in early gnathostomes [35, 48, 77]. The evolutionary process that has modified the lower jaw articulation is intimately related to the development of the mammalian hearing system. The jaw-opening reflex is essential for the development of the secondary cartilage capping the condyle; this condylar cartilage forms the articulation with the squamosal bone to create the definitive articulation of the lower jaw with the skull.

10.3.1 Molecular Regulation of Mandibular Development

Of the genes involved in neural crest cell formation, migration and differentiation, most modulate mandible development. Mutation or alteration of their expression can result in mandibular defects. This is also true for the genes expressed by the surrounding tissues, which influence the neural crest cell development in the mandibular pharyngeal arch.

Other factors influencing bone development and mandible patterning are Sonic hedgehog (Shh), Fgf8, Bmp4, and endothelin-1 signaling. These factors, expressed by the mandibular arch ectoderm or pharyngeal pouch endoderm on the underlying neural crest cells, predominantly act in the course of early mandible development.

Shh signaling is essential for the survival of the neural crest cell population that forms the mandible [90]. The absence of Shh signaling by targeted deletion in the mouse results in several craniofacial defects, including holoprosencephaly. Most Shh-negative embryos die early during embryonic development. It is difficult to establish the function of *Shh* in neural crest cells and mandible development [37]. McMahon et al. conditionally inactivated *Smo*, thus blocking the transcription of Shh-dependent genes [131]. This allowed Shh signaling to proceed normally in other mouse tissues [90]. The neural crest cell-derived craniofacial skeleton and the mandible were greatly reduced in size because of excessive death by the neural crest cells. These results demonstrate that Shh plays an essential role in neural crest cell survival.

Bone morphogenetic protein 4 (Bmp4) and fibroblast growth factor 8 (Fgf8) are both expressed by the mandibular arch ectoderm. *Bmp4* is expressed more distally and induces the ectodermal expression of the homeobox transcription factor, *Dlx2* (Fig. 10.2) [209, 224, 225]. *Fgf8* is expressed more proximally, inducing the mesenchymal expression of *Dlx2,* along with *Lhx6* and *Lhx7*. It appears that each factor represses the other, creating a proximo-distal boundary in the pharyngeal arch ectoderm that can be defined by the mutually exclusive domains of *Dlx2* expression. The absence of Bmp4 signaling

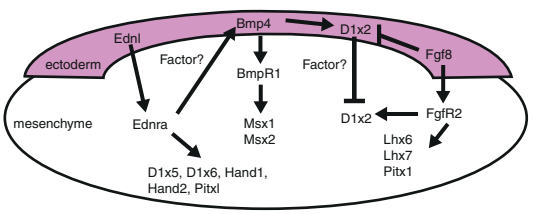

Figure 10.2. Complex mandibular patterning pathways that regulate development in the first pharyngeal arch.

is lethal during early embryonic development [235]. When Bmp4 was conditionally inactivated in the pharyngeal arch ectoderm and pharyngeal pouch endoderm [120], severe mandibular defects resulted, and the level of expression correlated with that of the remaining *Bmp4* expression. In embryos with minimal *Bmp4* expression, only the distal mandible development is affected, as evidenced by the absence of incisors. Embryos with near or total loss of *Bmp4* expression in the mandibular arch have no mandibular structure except for small, indeterminate intramembranous bones proximal to the squamosal bone. These findings indicate that mandibular development depends on different Bmp4 thresholds, as reflected during the expression of the genes *Msx1* and *Msx2* in the underlying crest cells and the expression of *Fgf8* in the ectoderm [120]. With less *Bmp4* expression, more *Fgf8* is expressed in the ectoderm, with the expression expanding distally. Bmp4 may therefore play a role in neural crest cell patterning and survival [87, 187, 188]. *Msx1*-null mice are born with mild distal mandibular defects: the incisors and molars are missing, and the alveolae normally encasing the teeth are filled by membranous bone [187]. In the *Msx2* knockout mice, the distal mandible is severely malformed, and teeth may be absent [188]. In the compound *Msx1/Msx2* double-mutant embryos, the mandible is much shorter and the proximal mandible and TMJ formation are affected. In addition, distal mandibular defects are present; these may be due to additive gene effects [87]. Bmp4 signaling, through the activation of the *Msx* genes, is therefore essential for mandibular patterning and tooth formation. This indicates that Bmp4 also has a role in distal mandibular development.

Of interest are the increased rates of apoptosis in the *Bmp4* conditional knockout embryos, resulting from an upregulation of *Fgf8* expression [120]. Conditional inactivation of the *Fgf8* gene in the pharyngeal arch ectoderm leads to a massive loss of neural crest cells by apoptosis [223]. However, Fgf8 is also a patterning factor that regulates the expression of mesenchymal genes, such as *Dlx2*, *Lhx6*, and *Lhx7* as well as the oral-aboral polarity [209, 224, 225]. An *Fgf8*

hypomorphic allele has been used to circumvent the problem of neural crest cell loss; the expression of this hypomorphic allele is lower than that of the wild-type allele, but is sufficient to prevent early embryonic death [60]. Embryos with one null and one hypomorphic *Fgf8* allele exhibit micrognathia, but the shape of the mandible is fairly normal [60]. However, the development of more proximal structures is affected when the TMJ fails to form and the mandible fuses with the squamosal bone and maxilla. This proximal transformation suggests that Fgf8 is also a factor patterning the lower jaw. Fgf8 patterning function is probably mediated by *Pitx1*, another gene regulated by the growth factor [209]. The distal mandible of *Pitx1* mutant mouse embryos is normal. However, the proximal mandibular structures are severely malformed, with most of the ramus missing. This is consistent with the role of Ffg8 in proximal mandible patterning [114].

Endothelin signaling also plays an important role in the patterning of the lower jaw bone. Endothelin-1 (Edn1) is secreted by the mandibular arch ectoderm, core paraxial mesoderm, and pharyngeal pouch endoderm, and binds to the endothelin-A receptor (Ednra) expressed in the neural crest cells (Fig. 10.2) [39, 179, 180]. Initially thought to be a signaling mechanism that guides the neural crest cell migration to the pharyngeal arch, the inactivation of either gene revealed that endothelin signaling is instead essential for inducing a mandibular identity in the neural crest cells [179]. In the absence of endothelin signaling in the mouse, the lower jaw undergoes transformation into upper jaw-like structures, including duplication of the maxilla, palatine, and zygomatic arch bones [179, 180]. This homeotic transformation may be an atavism, because the lower jaw is a mirror image of the upper jaw, as observed in more primitive animals (e.g., sharks) or in evolutionary ancestors of mammals [47, 48]. This transformation is caused by the downregulation of the expression of the mandibular genes (i.e., *Dlx5*, *Dlx6*, *Hand1*, and *Hand2*) in the first pharyngeal arch and the gain of maxillary gene expression (i.e., *Wnt5a*, *Dlx1*, and *Dlx2*) in the same arch [179]. A similar phenotype was observed in *Dlx5/Dlx6* knockout mouse embryos; therefore, the regulation of homeobox genes is essential for lower jaw patterning [17, 47]. Recent data suggest that endothelin signaling is the earliest patterning mechanism for the lower jaw, probably upstream of Bmp4 and Fgf8, inasmuch as the expression of the *Bmp4* and Fgf8-dependent gene *Pitx1* is affected in the absence of endothelin signaling [179, 180, 185, 186].

10.3.2 Patterning of the Mandibular Neural Crest Cells

Structural patterning of the body is associated with the regulation of the homeobox genes, and the mandible is no exception. The neural crest cells populating the mandibular arch are also referred to as the "Hox-less crest cells," as they are not influenced by *Hox* genes prior to their migration [43, 115]. This absence of *Hox* gene influence is essential for their patterning. As revealed by the inactivation of the *Hoxa2* gene in the mouse, abrogating the gene expression characterizing the second pharyngeal arch results in the duplication of the first arch structures (malleus and incus), instead of the formation of second arch structures [66, 173]. As the expression of the Hox genes is linked to retinoic acid signaling, it is not surprising that similar phenotypes were observed in different retinoic acid receptor mutant mice [121, 122, 129, 134]. Thus, in the absence of Hox signaling, second arch neural crest cells behave like first arch crest cells; this suggests that the expression of *Hox* genes in the first arch represses a mandibular phenotype. To determine whether the *Hox* genes inhibit lower jaw patterning, ectopic expression of the *Hoxa2* genes in the mandibular arch was studied in chicken and Xenopus embryos [68, 156], in which the mandibular structures were transformed into second arch-like structures. This mandible-to-hyoid homeotic transformation indicates that the neural crest cells carry prepatterning information; therefore, the absence of Hox signaling is essential to maintain mandibular identity. On the other hand, neural crest

cells have absolute plasticity, based on neural crest transplant experiments [184, 221]. The findings indicate that neural crest cells do carry some prepatterning information. Interspecies transplantation of similar axial neural crests between duck and quail embryos [190] demonstrates the presence of prepatterning information. In these chimeric birds, the quails develop a duck bill, whereas the ducks develop a quail beak. This not only shows that the crest cells carry prepatterning information, but that this prepatterning cannot be changed by the surrounding environment. Structural patterning is, however, associated with the expression of the homeobox genes. As no *Hox* gene is expressed early in the mandibular arch crest cell, other homeobox genes must compensate to establish mandible patterning, a function that is due to the *Dlx* genes.

Dlx genes (like *Hox* genes) are duplicated and found in tandem in the genome. They are expressed along a proximo-distal gradient, with *Dlx1* and *Dlx2* more proximal, *Dlx5* and *Dlx6* more distal, and *Dlx3* and *Dlx4* in between [47, 48]. On the basis of the gradient, it seems likely that Dlx1 and Dlx2 regulate the patterning of the more proximal structures, with Dlx5 and Dlx6 patterning the more distal structures. Inactivation of the gene encoding *Dlx2*, or the genes encoding *Dlx1* and *Dlx2*, has shown that the two genes are essential for the patterning of the upper jaw; their absence leads to palatine, maxillary, and zygomatic defects [164, 165]. The duplication of the proximal structures (e.g., incus) of the mandibular arch led surprisingly to the malformation of the middle-ear ossicles. This duplication implies that other genes are involved in the patterning of the middle-ear structures and the mandible.

The mandibular patterning of the first arch neural crest cells is absent when both *Dlx5* and *Dlx6* are inactivated, with the lower jaw replaced by upper jaw-like structures [17, 47]. This homeotic transformation is caused by the downregulation of genes that are normally expressed in the mandibular arch, such as *Hand2*, and the upregulation of genes associated with maxillary development, including *Dlx1* and *Dlx2*. This transformation is also an atavism, as described earlier. Axial patterning requires retinoic acid signaling, and the *Hox* genes are involved; in the mandibular neural crest cells, this interaction is provided by the endothelin/Dlx pathway [179, 180], a pathway also essential for the expression of transcriptional factors like *Hand1* and *Hand2*. Dlx6 directly activates the expression of *Hand2* by binding to the enhancer that drives the expression in the mandibular pharyngeal arch [36]. The two bHLH factors, Hand1 and Hand2, are essential for early embryonic development, as *Hand1*-null and *Hand2*-null embryos die at embryonic ages 8.5 or 10.5 days, respectively [56, 208].

In mice whose *Hand 2's* endothelin-dependent pharyngeal arch enhancer was removed by targeted deletion, the mandible is hypoplastic and porotic, and malformations are observed in the distal segment and in the angular process [62, 239]. These defects are consistent with the expression pattern of the Hand2 gene in the mandibular arch [178]. When the expression of both *Hand2* and *Hand1* genes is reduced, more severe distal defects are observed [12]. Both branches of the mandible are fused at the symphysis, and only one incisor forms; in other words, Hand1 can partially compensate for the loss of Hand2 function. The most interesting phenotype in the pharyngeal arch-specific *Hand2* mutant embryos is the porotic bone. This phenotype is caused by premature ossification of the mandible, with the depletion of the pool of crest-derived mesenchymal cells that differentiate into osteoblasts [62]. This premature ossification is caused by the loss of the Hand2 physical repression on the transcriptional activity of Runx2, a master regulator of bone differentiation. However, according to Ruest and Clouthier [180], the later expression of Hand2 in the mandibular arch is independent of endothelin signaling. This raises the question of what regulates Hand2 expression after activation by the endothelin/Dlx pathway and what regulates the onset of ossification in the mandibular arch. Conceivably, this is Bmp signaling. Endothelin signaling is intermingled with Bmp4 signaling [179]. Possibly, an unknown factor(s), released by the neural crest cells,

influences ectodermal gene expression. This uncertainty reflects the complexity of the signaling mechanisms that regulate mandibular patterning and development.

Endothelin signaling appears to be one of the earliest patterning mechanisms to establish a mandibular identity in the neural crest that populates the first arch, and does so by regulating *Dlx5*, *Dlx6*, *Bmp4*, *Pitx1*, *Hand1*, and *Hand2* expression [179, 180, 185, 186]. What is more, without endothelin signaling, the mandibular identity is lost and upper jaw-like structures develop in place of the mandibular arch. These findings indicate that endothelin signaling may repress upper jaw development and induce mandibular development ectopically. The restricted expression of Edn1 may explain why this situation does not occur, inasmuch as Ednra is expressed in all head neural crest cells [179, 186]. Kurihara's group in Japan produced transgenic mice that ectopically expressed the edn1 ligand. This caused activation of the Ednra receptor signaling in all head neural crest cells, and not only in those in the pharyngeal arches [185]. In the transgenic embryos, the maxilla is transformed into lower jaw-like structures, including Meckel's cartilage and a mandible-like formation. This homeotic transformation is accompanied by expression in the maxillary prominence of the lower jaw markers, *Dlx5*, *Dlx6*, *Pitx1*, and *Hand2,* a transformation that is mostly visible in the Hox-less-derived neural crest cells, with no effect on the development of the lower pharyngeal arch structures. As mentioned earlier, *Hox* genes are essential for the development of neural-crest-derived structures in the lower pharyngeal arches with the absence of *Hoxa2* expression causing an anterior-to-posterior transformation. The ectopic expression of *Hoxa2* in the Hox-less mandibular arch causes a posterior-to-anterior transformation [66, 68, 156,173]. The ectopic activation of endothelin signaling is reminiscent of the posterior-to-anterior transformation with *Hoxa2*. Retinoic acid regulates the expression of Hox homeobox genes in the posterior arch structures; this regulatory function in anterior structures is probably assumed by edn1 functions.

10.4 Temporomandibular Joint

10.4.1 Initiation as a Secondary Cartilage

All movements of the mandible take place at the articulation of the mandible with the cranium, the temporomandibular joint (TMJ). These bilateral synovial joints have an interesting developmental history that makes them different from the joints of the limbs. The general structure of the TMJ resembles that of most synovial joints, but the cartilage capping the mandibular condyle (mandibular condylar cartilage or MCC) is a *secondary cartilage* [16], a term indicating that the morphogenesis of the TMJ and its components begins after the bone of the mandible is present and after analogous joints in the limbs are formed. For the most part, the secondary cartilages are transient, occurring in numerous locations in the developing craniofacial complex [231]. They persist postnatally in the mandibular condyle, in the angular process of the mandible and the intermaxillary suture [82, 160]. The primary cartilages of the limbs typically arise via interaction between the mesenchyme and epithelium [75], while secondary cartilages typically develop in response to local biomechanical stimuli [16, 74]. The first sign of the future MCC, adjacent to the intramembranous bone of the mandible, is a condensation of the alkaline phosphatase-positive cells that are continuous anteriorly with the periosteum of the mandible [199]. Even though these cells are osteoblastic in nature [200], they seem to be bipotent, becoming chondrogenic when mobility and loading are normal and osteogenic, when motion is limited or loads are low or absent [82, 161]. This is reflected in the fact that cells in the MCC anlage express not only mRNA for osteogenic lineage markers (e.g., *collagen*, *Runx2*, and *Osterix)*, but also mRNA for *Sox 9*, a marker for chondrogenic differentiation [201]. Secondary cartilages such as the MCC are also characterized by very rapid differentiation of chondrocytes to hypertrophic chondrocytes, with extensive overlap between collagen types II and X. This differs from the stratification in different zones of the growth plate of limb cartilages [28, 61, 198].

10.4.2 Stages of TMJ Morphogenesis

Human TMJ morphogenesis does not commence until the embryo is 7 or 8 weeks old. By that time, which is called the *blastematic stage* [135], the major joints of the limbs are fully formed, and their structure resembles that of the adult joint [65, 139]. During this stage, the mesenchymal cells condense, such that the mandibular condyle and the temporal bone becomes recognizable [13, 117, 135, 226]. The condylar blastema grows dorsally and cranially, eventually to approximate the separate temporal blastema [14, 109]; this process is not completed until around 12 weeks [14, 242]. The mesenchyme located between the condylar and temporal blastemata comprises a third blastema that is thought to give rise to the articular disc [135, 226]. It is important to emphasize that the condylar condensation has no connection to primary cartilages, such as Meckel's cartilage or the cartilage anlage of the malleus (a middle ear ossicle). Premyoblasts that form the lateral pterygoid muscle appear around 6–7 weeks, and by 7 weeks, muscle fibers appear to attach to the condylar mesenchymal condensation [114, 117, 151].

During weeks 9–11 in utero, known as the *cavitation stage*, cells in the center of the condylar blastema differentiate into chondrocytes [14, 117]. At the same time, the mesenchyme separating the condylar and temporal components becomes denser; this signals a more definitive anlage of the future articular disc. Coincident with this, small clefts between the disc anlage and the condyle can be observed [151, 159, 226]. These events reflect the initial formation of the inferior joint cavity, which is typically completed through the coalescence of the clefts by week 10. Interestingly, the upper joint space does not become evident until week 11 or 11.5 [226]. By week 12, two parts of the lateral pterygoid muscle appear: an upper part, in contact with the medial aspect of the disc, and a lower part, in contact with the condyle [151].

The *maturation stage* of TMJ development [135, 226] begins in week 12. All the major components of the TMJ, present in a form that approximates their eventual postnatal structure, commence a period of continued growth and consolidation. The disc is comprised of densely packed fibroblasts and exhibits a thinned area centrally, with thickening laterally and medially [63]. The joint capsule which, at 9–11 weeks, is a stained zone near the periphery of the mesenchymal block [135, 226], becomes clearly evident by week 14 [151]. The temporal component that has been described as convex or flat during the cavitation stage [135, 167, 226] now assumes a slightly concave shape that is reminiscent of what is seen postnatally, although no true eminence is present. The development of the human TMJ is schematized in Fig. 10.3.

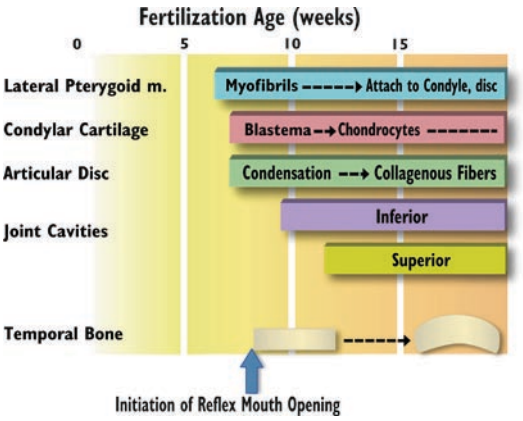

Figure 10.3. Timeline of human TMJ development showing changes in different joint components.

Human fetuses can open their mouth beginning at the age of 8.5 weeks; this is a component of total contralateral head, trunk, and rump flexion [84]. After 11 weeks, mouth-opening reflexes are accompanied by progressively fewer trunk and extremity movements; by 15 weeks, mouth opening occurs without head or other movements [84]. As the superior and inferior joint cavities arise between 10 and 11.5 weeks, cavitation in the TMJ may be stimulated by these early jaw movements. However, the reflex mouth opening reported at 8.5 weeks is independent of the TMJ, which is only in the process of formation at this time. Some investigators [11, 135] have suggested that these early movements take place at the malleus/incus joint at the rostral end of the Meckel's cartilage. If this is the case, it is intriguing to speculate whether initiation of chondrogenesis in the condylar cartilage at 9 weeks is engendered by these early reflex movements. This may be true because the ex utero

limitation of jaw movements in mouse fetuses are observed to reduce growth at the condylar cartilage [73]. Similarly, the absence of fetal swallowing in human newborns is strongly associated with micrognathia [195].

10.4.3 Postnatal Growth and Maturation

Unlike the limbs, in which growth and articulation are carried out by separate growth and articulation cartilages, the MCC incorporates both the functions in a single tissue. The MCC is an active site of endochondral growth during prenatal and early postnatal life (Fig. 10.4), yet its thickness decreases to two-thirds by 6 months of age in humans [32]. The MCC continues to grow at an ever-attenuating rate until the mid to late teens [237]; notwithstanding that, during puberty, it is subject to increasing loads owing to muscle hypertrophy. In the rat, the percent of cells undergoing mitosis in the MCC declines with age to 47% of the value at weaning at puberty, and to 7% by full adulthood [230]. A solid subchondral plate of bone seals off the cartilage from the medullary spaces by 20 years of age [86, 215], effectively preventing further endochondral ossification by denying blood vessels from the marrow access to the deeper layers of the cartilage. Over the ensuing years, the near- or complete loss of the prechondroblastic (mitotic) cells and reductions in overall cellularity, including chondrocytes, transform the MCC into a relatively homogeneous fibrocartilage [123, 124]. This completes a cycle in which the MCC, initially a site of rapid growth with minimal loading, takes on both growth and articular functions, and is ultimately transformed into a purely articular (fibro) cartilage. It has only recently become apparent that the unusual developmental history of the MCC and the resultant differences in its histological structure and composition from limb cartilages give rise to material properties and growth characteristics that must be taken into account in attempts to develop constructs that mimic native TMJ tissues [8, 232] or to stimulate the overall length of the mandible beyond its normal growth potential [166].

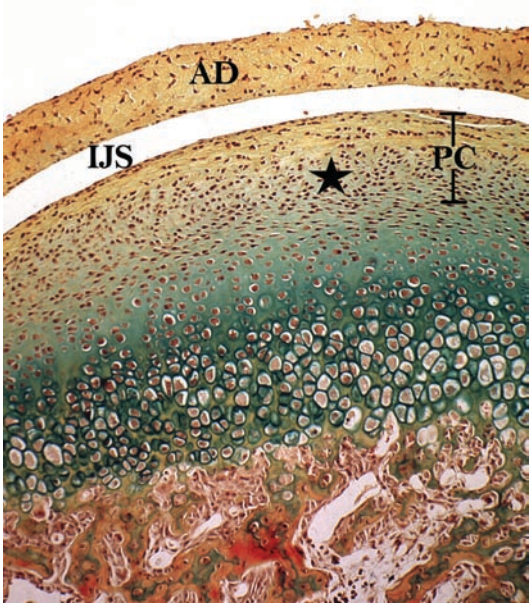

Figure 10.4. Mandibular condylar cartilage (MCC) in a rapidly growing rat. AD, articular disc; IJS, inferior joint space; PC, perichondrium. The star designates the inner layer of the perichondrium where cells in the MCC undergo division, when compared with the deeper-lying chondrocytes in a growth plate. Attwood stain.

10.5 Palate Development

10.5.1 Classic Theories of Palatal Morphogenesis

The palatal structures are composed of the cranial neural crest-derived mesenchyme and pharyngeal ectoderm [79, 99, 147]. The epithelia that cover the palatal shelves can be divided into oral, nasal, and medial edge epithelia (MEE). The nasal and oral epithelia differentiate into pseudostratified and squamous epithelia, whereas the MEE is removed from the fusion line (Fig. 10.5).

The secondary palate originates as an outgrowth of the maxillary prominences on or about embryonic day 11.5 in the mouse (E11.5-m) and 6 weeks post-conception (p.c.) in humans (6 week-h). The palate shelves initially grow vertically along the sides of the tongue (E13.5-m; p.c. 7 week-h), and then rise above the tongue as

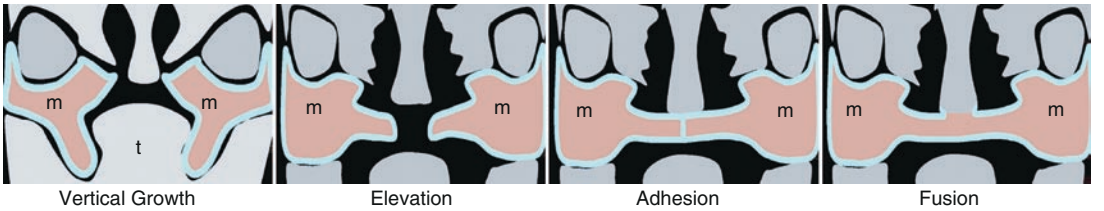

Figure 10.5. Coronal view of a normal palate shelf and the key stages of mouse palatal development. At days E12-E13 in the gestation of the mouse, the palatal shelves grow downward along the tongue (t). At E13-E13.5 days, the palatal shelves become elevated above the tongue. At E14.5, the palatal shelves adhere to each other in the midline. After E15.5 days, the MES completely degrades and the palate fuses. (Reprinted with permission from Yu et al. [241]).

the latter drops in the oral cavity, owing to the forward and downward growth of the mandible (E14.0-m; p.c. 8 weeks-h). With continued growth, the shelves appose at the midline (E14.5-m; p.c. 10 weeks-h) and eventually fuse (E15.5-m; p.c. 13 week-h) [144]. Numerous genes that are similar in mice [241] and humans [26, 144, 196] are expressed (Table 10.1) during palatal development.

During fusion, the epithelium covering the tip of opposing palatal shelves adheres, intercalates, and thins into a single-layer midline epithelial seam (MES) [147]. The disintegration of this seam results in the confluence of the palatal mesenchyme. Tremendous interest has arisen in the cellular mechanisms underlying MES degradation. The epithelial-mesenchymal transition (EMT) is one of the proposed models that regulate the MEE cell fate [79, 96, 99, 147, 202]. However, other mechanisms have been proposed, such as apoptosis [44, 141, 217], in which all the MEE cells are thought to die during fusion. Alternatively, the MES cells may disappear by migrating from the midline toward the nasal and oral epithelia [29, 94]. Conceivably, apoptosis, migration, and EMT may all occur [133, 141, 147]. The fusion of the external surface of the bilateral maxillary processes with the nasofrontal prominence in the chick is similar to palatal fusion (Fig. 10.6) [211]. The outer periderm layer dies through apoptosis, and the lateral edge epithelium of the intermaxillary segment of the nasofrontal process fuses with the medial edge epithelium of the external maxillary process to form a seam that transitions to a confluent mesenchyme (Fig. 10.6) [211].

10.5.2 Molecular Mechanisms in Embryonic Palatal Development

The genes that are expressed to induce the different stages of palatal development have been examined through observations of human and animal oral facial clefts. Cleft lip with or without cleft palate (CL/P) is a complex trait caused by many genes and environmental factors [143].

The failure of the palatal shelf formation is a rare, but severe defect. Signaling networks between the epithelium and mesenchyme involve signaling molecules and growth factors such as Shh, members of the transforming growth factor β (TGFβ) super family including BMPs and TGFβs, FGFs, and their receptors (FgfR), as well as other effectors and targets [144]. FGF signaling during early palatal development alters cell proliferation within both mesenchyme and epithelium in the palatal shelves, and induces an increase in apoptosis within the epithelium. In Fgf10 and FgfR2b mouse mutants, the initial development of the palatal shelves was altered and the pups had a fully developed cleft palate (CP) [170]. By signaling via its receptor [170] in the palatal shelf epithelium, the mesenchymally derived Fgf10 supports epithelial proliferation and survival and induces the expression of Shh within the epithelium. Shh, in turn, signals to the mesenchyme and stimulates cell proliferation. Signaling activities are generally subject to tight spatiotemporal control and, in many instances, too much or too little signal is detrimental to the developing organ. This situation is well illustrated in anomalies caused by deregulated hedgehog (Hh) and FGF signaling [149, 171]. Fgf10/FgfR2b activity plays a crucial role during

Table 10.1. Syndromic genes associated with cleft and palate

Syndrome	Clinical features	Genes	Reference
Apert syndrome	AD; high arched palate, bifid uvula, and cleft palate	FGFR2	[72, 130]
Bamforth–Lazarus syndrome	AR; hypothyroidism, athyroidal, CPO, choanal atresia, spiky hair	FOXE1	[33, 72]
Branchio-oculo facial syndrome	AD; pseudocleft of the upper lip resembling a poorly repaired cleft lip	TFAP2A	[72, 137]
Down syndrome	Macroglossia, microstomia, atlantoaxial subluxation	Duplication of portion of chromosome 21	[98]
Ectrodactyly-ectodermal dysplasia-cleft syndrome	AD; triad of ectrodactyly, ectodermal dysplasia, and facial clefting	P63	[72]
Fetal alcohol syndrome	Disorder characterized by a pattern of minor facial anomalies, prenatal, and postnatal growth retardation	Alcohol dehydrogenase 1B	[1, 69]
Goldenhar syndrome	Oculo auricular vertebral dysplasia; AD; incomplete development of the ear, nose, soft palate, lip, mandible	Pericentric inversion of chromosome 9	[108, 230]
Hereditary lymphedema-distichiasis syndrome	AD; lymphedema of the limbs, double rows of eyelashes, cardiac defects, and cleft palate	FOXC mutations	[52]
Kallmann syndrome	AR disorder; Hypogonadotropic hypogonadism and anosmia	FGFR1 mutations	[50, 72]
Margarita Island ectodermal dysplasia [71]	AR; unusual facies, dental anomalies, syndactyly, and cleft lip/cleft palate	PVRL1 (nectin-1) mutation	[72, 212]
Pierre Robin sequence	AD; triad of micrognathia, glossoptosis, and cleft palate	Loci 2q24.1–33.3, 4q32qter,11q2123.1, and17q2124.325.1	[40, 162]
Smith–Lemli-Opitz syndrome	AR; defects in cholesterol biosynthesis, growth retardation, dysmorphic facial features including CLP/CPO, postaxial polydactyly	DHCR	[72, 142]
Stickler syndrome	AD; midface hypoplasia, micrognathia, Pierre robin sequence, retinal detachment and early cataracts, deafness, hypermobility of joints	Col11A1, Col11A2, Col2A1	[206, 234]
Treacher-Collins syndrome	AD; craniofacial deformities such as: downward slanting eyes, micrognathia, conductive hearing loss, underdeveloped zygoma	Mutation in TCOF1 gene at chromosome 5q32-q33.1	[49]
van der Woude syndrome	AD; cleft lip palate, distinctive pits of the lower lips, or both	IRF 6 (interferon regulatory factor 6) mutations	[72, 111]
Velocardiofacial syndrome	AD; cleft palate, heart defects, abnormal facial structure, and learning problems	Chromosome 22q11 microdeletion	[45, 140]
Unnamed syndrome	CL/P and hereditary diffuse gastric cancer	CDH1	[245]
Unnamed syndrome	Chromodomain helicase DNA-binding proteins; CL/P in Charge syndrome	CHD7	[182]
Unnamed syndrome	Bilateral CL/P, colobomas of the optic nerve and retina, agenesis of the corpos callosum. Dysphagia, reduced esophageal peristalsis	PAX 9	[72] #5801
Unnamed syndrome	X-linked mental retardation and CL/P	PH8	[2]
Unnamed syndrome	Holoprosencephaly 7, a spectrum of forebrain and midline anomalies and midline CL	PTCH	[72]
Unnamed syndrome	CPO, craniofacial anomalies, osteoporosis, and cognitive defects	SATB2	[72]
Unnamed syndrome	Holoprosencephaly, a spectrum of anomalies ranging from severe (cyclopia) to subtle midline assymetries CL/P part of the spectrum	SHH	[86, 180]
Unnamed syndrome	Anomalies with most features of DiGeorge/velocardiofacial syndromes: CPO, thymus, and parathyroid gland hypoplasia, vertebra, facial, and cardiac outflow anomalies	TBX1	[86, 305]

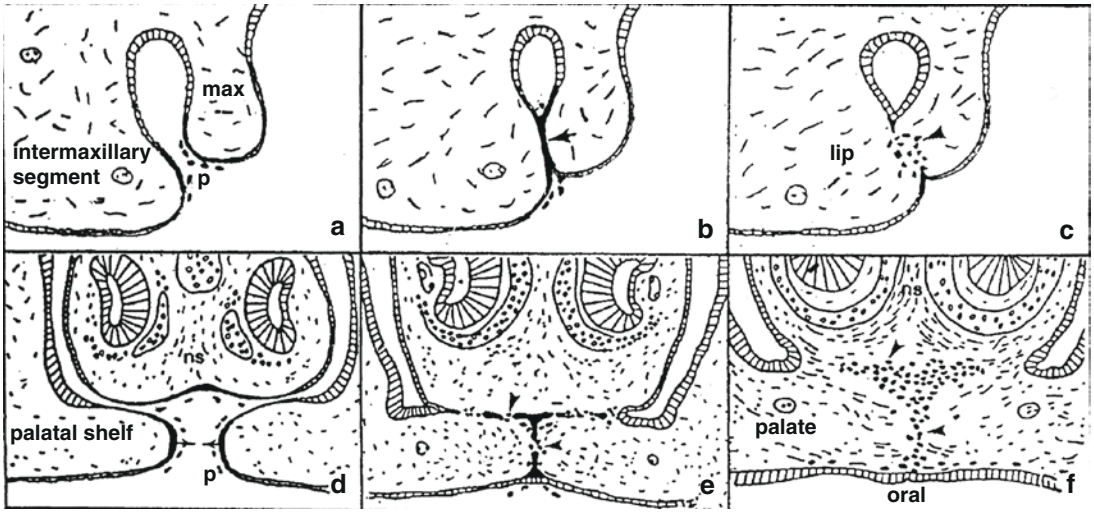

Figure 10.6. Comparison of the morphogenesis of the upper lip (**a**–**c**) with that of the palate (**d**–**f**). After the bilateral maxillary processes (max) fuse externally with the intermaxillary segment, the resulting epithelial seam (*arrow*, **b**) gives rise to mesenchyme (*arrowhead*, **c**) to produce a confluent lip. At a later time, the palatal shelves arising internally from the maxillary processes fuse with each other (*arrows*, **d**) and with the nasal septum (ns) above them, creating an epithelial seam that transforms to mesenchyme (*arrowheads*, **e**) to produce the confluent palate (*arrowheads*, **f**). *p* sloughed periderm cells. Reprinted with permission from Griffith and Hay [70], at www.dev.biologists.org, and Sun et al. [211] copyright 2000, with permission from Elsevier).

palatogenenesis, but appears subject to the tight spatiotemporal regulation prevailing in mice lacking *Shox2*. *Shox2*-mutant mice develop a very rare type of CP, where the soft palate is intact, but the hard palate has a cleft [80]. This condition is also found in humans. Abnormal proliferation and apoptosis may cause the cleft. Yet, many factors that play a role in palatogenesis including *Msx1, Bmp4, Pax9, Lhx8, Osr2*, *Tgfβ3*, and *Jag 2* are normally expressed [80]. In contrast, Fgf10 and Fgfr2b are expressed at ectopic sites within the mesenchyme of Shox2-mutant mice [240]. These studies emphasize the importance of the precise timing and sites of signaling activities that are necessary for normal development.

The mutation of activin-βA causes a severe facial primordial development defect, which may be responsible for the retardation of palatal shelf development and complete CP. In addition, other genes, including *Msx1, Lhx8, Shox2*, and *Osr2,* assume important roles in the palatal shelf growth. The targeted mutation of these genes in mice generates CP; this indicates that these factors are essential for palatogenesis [240].

Under normal conditions, the palatal shelves do not fuse with other oral structures. However, in mice that do not express Fgf10, the palatal shelf epithelium fuses with the tongue and mandible [170]. The loss-of-function mutations of Fgf10 result in anterior palatal shelf fusion with the tongue. At the same time, the middle and posterior palatal shelf regions adhere to the mandible, thus preventing the elevation of the palatal shelf [6]. There is a severe reduction in the *Jagged 2* expression, encoding a ligand for the Notch family receptors and the ectopic production of Tgfβ3 in the nasal epithelia of mutant mice. On the basis of the analysis of *Jag2* mutant embryos, it is apparent that Jag2-Notch signaling prevents inappropriate palatal shelf adhesion to other oral epithelia through its control of the oral epithelial differentiation. Mutations in TBX22 have been reported in families with X-linked CP and ankiloglossia [24, 128, 236]. *Tbx22* is expressed in the developing palate and tongue in mice; this suggests an important role in regulating tongue and palate development.

The role of Tgfβ3 during palatal fusion has been studied extensively [58, 158]. For fusion to occur, the MEE must adhere to the palatal shelf. TGfβ3 is expressed in the MEE before and during fusion, and mediates MEE adhesion of the

opposing palatal shelves through filopodia. E-cadherin is required for fusion, whereas the filopodia seem to be crucial for the proper alignment and guidance of cell sheets that are fated to fuse, but not for the fusion itself [191]. Tgfβ3 is implicated in controlling the remodeling of the extracellular matrix through regulation of the expression of matrix metalloproteinases (MMPs), MMP13, MMP2, and the tissue inhibitor of metalloproteinase (TIMP)-2 [19]. TGFβ3 signaling functions in the MEE to mediate epithelial–mesenchymal interactions leading to changes that regulate palatal fusion. For example, EMT of the MES may constitute the major mechanism that underlies the disappearance of the MES, which generates mesenchyme continuity and prevents palatal clefts [146]. The establishment of the concept of EMT as the prevailing mechanism of MES disappearance has led to studies attributing roles to various molecules including Tgfβ3, Lef1, Smad, RhoA, phosphatidylinositol 3-kinase (PI-3 kinase), MMPs, Twist, and Snail [97, 99, 145]. In *Tgfβ*- or *Egfr*-mutant mice, the fate of MEE cells is altered [95, 136], and the MEE cells fail to undergo apoptosis and persist along the midline, thereby preventing normal fusion.

10.5.3 Ossification of the Palate

Palatal fusion indicates the ossification process that forms the hard palatal tissues in the anterior two-thirds of the palate. This entails the successful fusion of three embryonic structures, namely, the lateral edges of the primary palate with the two anterior edges of the secondary palate. This process requires the synchronization of shelf movements, together with the growth and withdrawal of the tongue and the growth of the mandible and head [31]. Any form of disruption during the formative stages results in a pathological cleft.

A wide range of studies on craniofacial skeletal maturation has shown that the fusion of the palatal shelves along their length to form the midpalatal (MP) suture occurs in the course of the ossification of the maxillae and palatine bones before the development of mandibular condyle [80, 105, 152, 157]. Ossification is observed where mesenchymal cells condense, the surrounding tissue vascularizes, and cells differentiate into osteoblasts. A number of growth and differentiation factors are involved in this process, e.g., BMPs, core-binding proteins, FGFs, and Hh proteins. These interact with various signaling pathways to regulate the patterning of undifferentiated mesenchyme. Bmp-6 and the transcription factor Gli1 are also expressed during intramembranous bone formation [89, 103]. As in the craniofacial sutures, the MP and TP suture osteoblasts express Tgfβ1,2, and 3, while the suture cells primarily express Tgfβ3 [3, 4].

Cranial sutures are the growth sites for the neurocranium, with the dura mater providing the signaling molecules to regulate suture patency [153]. The MP and TP sutures have different structures and are not in contact with the dura mater. Opperman's group has proposed that these facial sutures are growth centers [6, 7] and that the nasal capsular cartilage produces signaling molecules that regulate the fusion of the MP and TP sutures (Fig. 10.7) [4]. The nasal cartilage maintains the TP sutures as growth sites with or without nasal cartilage. Conceivably, the nasal cartilage regulates mid-facial growth [4].

Cyclic forces constitute an effective mechanical stimulus for the regulation of osteogenesis and osteoclastogenesis in the growth of sutures in neonatal rats [229]. At the early stage of sutural expansion, when osteoblasts and fibroblasts have proliferated, application of tensile forces has led to a cyst-like zone at the conjuncture of the bony front and the sutural connective tissue. New bone is deposited along the nasal septum and at the front of the cyst until the new bone front is formed, with the original structure restored by the suture [119].

Utilizing the MP suture expansion in mice has provided insight into how mechanical stress modulates remodeling of bones and cartilage. The expansive force across the MP suture promotes bone resorption through the activation of osteoclasts and bone formation through the increased proliferation and differentiation of periosteal cells [83]. Similarly, when expansion by orthodontic wire was done in growing rats, the secondary cartilage underwent chondrogenic and osteogenic differentiation in the maxillary arch. These induced changes have been attributed to progenitor cells differentiating from chondroblast to osteoblast. When this occurs, many sutures

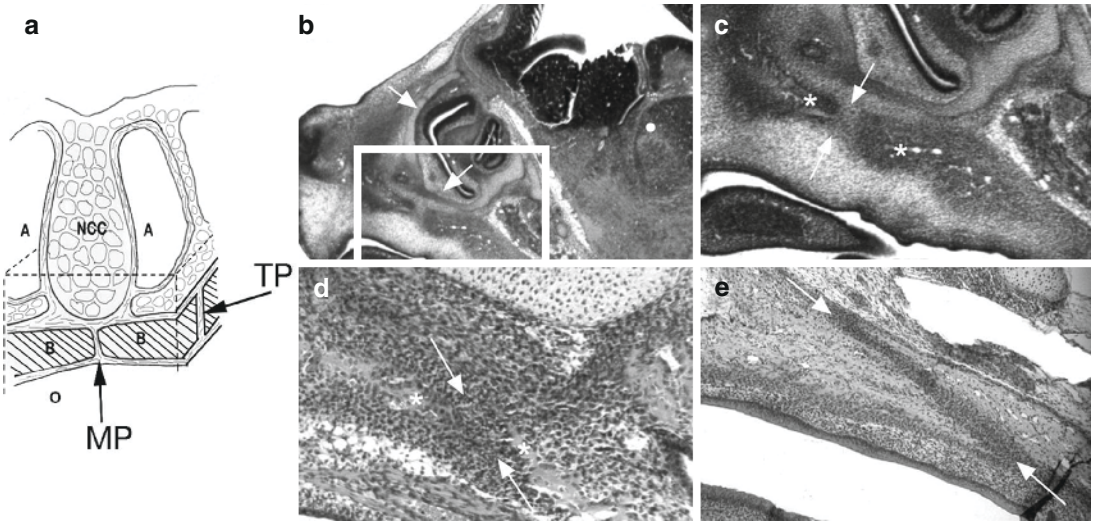

Figure 10.7. (**a**) Relationship between the nasal capsular (NC) cartilages and the transpalatal suture. *Dotted lines* indicate cut lines for removing the palate from the embryo and the NC cartilage from above the sutures. (**b**–**e**) Micrographs of parasagittal sections through fetal rat heads, showing the prenatal development of transpalatal (TP) sutures. (**b**) At E16, NC cartilages (*arrows*) can be seen directly above the presumptive TP suture region (in *box*). (**c**) High-power micrograph of the region in the box, showing the advancing palatal plate of the maxilla and horizontal plate of the palatal bone (*asterisks*) on either side of the presumptive TP suture (between *arrows*). (**d**) At E18, the advancing bone fronts (*asterisks*) overlap, creating a highly cellular suture blastema (between *arrows*). (**e**) By E20, an elongated TP suture (between *arrows*) continues to form as the bone fronts proceed to overlap. *A* airway; *B* shelves of maxillary bones; *MP* mid-palatal suture; *NCC* nasal capsular cartilage; *O* oral cavity; *TP* transpalatal suture. (Reprinted with permission from Adab et al. [3]).

temporarily form secondary cartilage. Histological observations at days 7, 10, and 14 have shown that intramembranous bone formation occurred at the boundary between the precartilaginous and cartilaginous cell layers, with osteocalcin detected in the calcified matrix [214]. The cellular events that take place at the MP suture cartilage expansion force are similar to what occurs when endochondral bone is formed at the boundary between the maxillary bone and cartilage. On the other hand, intramembranous osteogenesis appears at the internal aspect of the cartilaginous layer [107].

To stimulate new bone formation, rat organ cultures with distracted palatal sutures were treated with Bmp-7 and Nell-1 for 8 days in vitro. Nell-1 increased chondrocyte hypertrophy and endochondral bone formation, whereas Bmp-7 enhanced chondrocyte proliferation and differentiation in the distracted palates of the 4-week-old male rats. This study indicates that Nell-1 is involved in the rapid osteoblast differentiation in palate sutures [42]. When TGF-β1 was applied during the early stages of rat MP expansion, bone formed, presumably at the suture site [189].

10.5.4 Oral and Palatal Musculature and Related Deformities

Defects encompassed in overt cleft lip and palate range from the so-called microform clefts to complete unilateral or bilateral clefts of the lip and palate. The orbicularis oris (OO) muscle consists of many muscular fiber strata that are differently oriented and surround the mouth opening. At approximately 7 weeks p.c. in humans, the two maxillary prominences fuse with the medial nasal prominence; however, lip fusion is not complete until the epithelial seam disappears through EMT and/or apoptosis [31]. By 8 weeks p.c., there appears a dense, continuous band of mesenchymal cells that give rise to the OO muscle; discernible OO muscle fibers are present by 12 weeks and the complete OO muscle architecture is formed by 16 weeks [93]. Any delay in fusion may result in a subepithelial OO defect, such as the altered migration of the mesenchymal cells. Subepithelial (nonvisible) defects of the OO muscle represent the mildest form of cleft lip, constituting a part of the phenotypic spectrum of CL/P. This defect

is a ridge of tissue, resembling a scar on the upper lip along the philtrum [5].

Histological studies have shown that the above-described defects extend to the muscle fibers of the superior OO muscle. A method using high-resolution ultrasonography was developed to visualize the OO muscle noninvasively [148]. OO defects differ significantly between first-degree relatives of the CL/P individuals and controls, with the differences detected by ultrasound, consistent with the information from autopsies [127, 148]. Interestingly, in the Bmp4 knockout mouse, bilateral cleft lips can be seen at E14.5, even though this condition occurs only in 22% of newborns [93]. This suggests that the initial cleft lip is rescued or healed in utero, leaving only the subepithelial OO defect. Two individuals with OO defects were found to have mutations in BMP4, whereas none was found in the controls [150]. The strong evidence that OO discontinuities are part of the phenotypic spectrum of CL/P is important for estimating the clinical recurrence risk for families with members affected with CL/P.

The mildest form of CP is termed as a "submucosal CP." This is made up of a bifid uvula, palatal muscle diastasis, and a notch in the posterior surface of the hard palate [67]. Defects in the nasopharyngeal structure and/or function may lead to velopharyngeal incompetence (VPI). Although most VPI is caused by CP, the population prevalence of VPI owing to other causes is approximately 2.5% [20]. VPI may be caused by submucosal muscular defects of the levator veli palatini or musculus uvulae. Most of the soft-palate muscles are derived from myotome cells, which invade pharyngeal arch 4 and then migrate to the palate, with innervation from the vagus nerve. One muscle (tensor veli palatini) is derived from myotome cells that invade arch 1 and are innervated by the trigeminal nerve [213]. In the mouse, the tensor veli palatini, levator veli palatini, medial pterygoid, and lateral pterygoid muscles are myogenic fields, beginning with gestational day 15. The palatoglossus, palatopharyngeus, and musculus uvulae, however, are not yet in evidence [222]. The presence of these anatomical features in unaffected individuals may constitute an elevated risk for producing clefts in offspring [233].

10.5.5 Small Ubiquitin-Like Modifier (SUMO) Modification of Signaling Pathways in Palatogenesis

The molecular understanding of nonsyndromic (NS) CL/P is further complicated because penetrance may differ significantly when a given mutation is placed on different strains. This reflects the fact that CL/P pathogenesis is subject to both genetic and environmental modifiers. One posttranslational mechanism is due to the SUMO [157]. SUMO proteins are ubiquitously expressed throughout the eukaryotic kingdom [243]. SUMO1 shows strong expression in the medial edge epithelial of the secondary palate [7]. A translocation breakpoint interrupting SUMO1 was found in a patient with CLP [7]. This translocation defect is the cause of CL/P and has been confirmed in SUMO1-deficient mice [7]. Moreover, mutations in TBX22 profoundly affect sumoylation, with changes in this process at least partially responsible for loss of function [10]. Other SUMO targets include Smad4, Msx1, p63, Pax9, Eya1, and FGF signaling [157], a change in some of which may affect the SUMO pathway. Destabilizing the normal balance of expression and activity for genes such as TBX22, Msx1, SATB2, and p63 during early pregnancy is likely to constitute a risk for the occurrence of CL/P. On elucidating the relationship of environmental factors, the SUMO pathway and the networks of craniofacial genes influenced by posttranscriptional modification may be crucial to understanding the idiopathic forms of orofacial clefts.

10.5.6 Overview of the Genetic Etiology of Cleft Palate

Disturbances at any stage during palate development, e.g., defective palatal shelf growth, failed or delayed elevation, and blocked fusion, can result in CP [38, 54]. As one of the most common congenital craniofacial defects, CP occurs in approximately 1 per 750 live births in the United States [38, 59]. Clefts occur more frequently among Asians (about 1:400) and certain American Indians than in Europeans. Clefts are relatively less common among Africans and African Americans (about 1:1,500) [204].

The etiology of CL/P is due to a combination of genetic and environmental factors [72, 143]. Characterization of the genomic sequences will pinpoint variations in the stages of craniofacial morphogenesis. Classification of CL/P into syndromic (Table 10.1) and NS genes (Table 10.2) helps to understand the molecular and genetic mechanisms that affect these types of craniofacial defects [30, 35, 71]. In addition, there is strong evidence that environmental factors such as alcohol consumption, tobacco, and anticonvulsants increase the risk of CL/P [192, 238]. On the other hand, folic acid may have a protective effect on CL/P and neural tube defects [21, 25, 55, 88, 112]. Recent data from the National Birth Defect Prevention Network have shown that neural tube defects have decreased from 5/10,000 to less than 2/10,000 after the food supply was fortified with folic acid. This vitamin and the proteins that facilitate its uptake and metabolism may be candidate genes in craniofacial development [55, 113, 193, 216, 244].

10.6 Tooth Development

From an evolutionary perspective, mammalian dentition is considered as a segmental or sequentially arranged organ system, in which specific numbers of teeth are spatially organized along the linear axes of the jaws. Biochemical and developmental pathways that control the initiation of tooth development in different vertebrate species are remarkably conserved. For developmental biologists, the developing tooth model is useful for studying the modes of patterning and morphogenesis that determine the position, size, shape, and number during organogenesis. For stem cell biologists and tissue engineering experts, the forming and fully developed tooth is an attractive model that offers both challenges and opportunities. Adult teeth house reservoirs of postnatal or "niche" stem cells that differentiate into bone and dentin and other tissues. These cells have been utilized in tissue engineering to regenerate mineralized tissues [64, 197]. As the implantation of a bioengineered "test tube tooth" would not result in life-threatening consequences, there is burgeoning interest in the field of bioengineering to utilize the wealth of genetic knowledge about tooth development to regenerate whole tooth organs for replacement therapies. Understanding the basic principles that guide the development of teeth, one of the most complex mammalian organ systems, is critical for regenerative medicine.

Teeth develop in distinct stages that are easily recognizable under the microscope (Fig. 10.8). They are the lamina, bud, cap, and bell (early and late) stages of tooth development [218]. As the molecular events that transform the tooth bud into a fully formed mineralized tooth have become known, the following functional terms can be used to describe the developing tooth organ: initiation, morphogenesis, cell or cytodifferentiation, and matrix apposition. The first visible sign of tooth development, becoming evident around 5 weeks of human development and at embryonic day 11 (E11) in mouse gestation, is a thickening of the oral epithelium that lines the frontonasal, maxillary, and mandibular arches. At this stage, the *dental lamina* is inductive and can direct the mesenchyme to a tooth-forming mesenchyme. As development progresses, the dental lamina grows into a *bud* that is distinguished by a well-demarcated zone of condensed ectomesenchyme called the *dental papilla*. At this stage, the inductive or tooth-forming potential is transferred from the dental epithelium to the dental papilla. At the cap stage, further elongation occurs, and the ectodermal component is referred to as the *dental* or *enamel* organ. The enamel organ and dental papilla become encapsulated by the *dental follicle*, a thin layer of mesenchymal cells that separate the dental papilla from the other connective tissues of the jaws.

The transition from the bud to the cap stage marks the onset of crown formation. Similar to the signaling centers found in other tissues, the tooth organ utilizes the *enamel knot* as an important organizing center that initiates cuspal patterning [91]. In this region, a unique set of signaling molecules is expressed. They modulate the shape of the crown and the underlying dental papilla. The enamel knot undergoes programmed cell death or apoptosis after cuspal patterning is completed at the onset of the early bell stage. The dental organ then assumes the shape of a bell as cells continue to divide

Table 10.2. Nonsyndromic genes: interaction effects of genes and environmental risk factors on oral clefts

Gene	Functional role	Risk factor	References
Cytochrome P450 proteins (CYP) CYPIA1, CYPIA2, CYPIB1 CYP2E1	Highly polymorphic, having multiple functional alleles; role in detoxification; metabolism of endogenous morphogens in the developing fetus	Negative gene–smoking interaction effect	[81]
Epoxide hydrolase (EPHX)	Class of proteins that catalyze the hydration of chemically reactive epoxides into their corresponding dihydrodiol products		
EPHX	Plays an important role in both the bioactivation and detoxification of exogenous chemicals, such as PAHs, which are present in cigarette smoke	Negative gene–smoking interaction effect	[9, 81]
EPHX1 Y113H	Variant of EPHX 1 found in the fetus and maternal smoking	Positive gene–smoking interaction effect	[196]
Glutathione transferase gene family (GST)	Families of dimeric phase II enzymes that catalyze the conjugation of reduced glutathione with electrophilic groups of a wide variety of environmental agents		
GSTM1	Major gene detoxifying PAHs and widely studied in many disorders and cancers	Negative gene–smoking interaction effect	[196]
GSTT1	Expressed in a variety of tissues/organs, such as erythrocytes, lung, kidney, brain, skeletal muscles, heart, and small intestine; elevated expression profile at the craniofacial regions during embryonic development	Positive gene–smoking interaction effect	[196]
GSTP1	Major gene detoxifying PAHS; involvement in a variety of disorders and cancers. Major enzyme involved in the inactivation of cigarette smoker's metabolites; most important isoform at the embryonic and early fetal developmental stages	Positive gene–smoking interaction effect	[196]
GST A4/GSTM3	Two other types of GST gene family members	Positive gene–smoking interaction effect	[196]
Hypoxia-induced factor-1	Mechanism by which maternal smoking may affect embryonic development owing to the production of carbon monoxide that interferes with oxygen transfer to the placenta, or nicotine that constricts the uterine wall resulting in hypoxia	Positive gene–smoking interaction effects	[196]
Arylamine *N*-acetyltransferase gene family	N-conjugation of arylamine by the action of *N*-acetyltransferases (NAT), UDP glucoronosyltransferases (UGTs), or sulfotransferases (SULTS0) produces nontoxic compounds		
NAT 1	Expressed in many tissues, such as erythrocytes, bladder, lymphocytes, neural tissues, liver, and intestines	Negative gene–smoking interaction effects	[113, 203, 205]
NAT pseudogene (NATP1)	Pseudogenes identified, which are located at chromosome 8p23.1–8p21.3		[113, 203, 205]
NAT 2	Expressed in the liver and epithelial cells of the intestine	Positive gene–smoking interaction effects	[196]
Methylenetetrahydrofolate reductase (MTHFR) MTHFRC677T	Metabolism of folate by reducing methylenetrahydrofolate, primary donor for methionin synthesis. Variant of methylenetetrahydrofolate reductase	Positive gene–vitamin interaction effect Negative gene-smoking interaction effect	[18, 132]
Other metabolic genes			
NAD(P)H quinine oxidoreductase (NQO1)	Flavoenzyme that catalyzes two electron reduction of quinine compounds to hydroquinone and is inducible by oxidative stress, dioxin, and PAHS found in cigarette smoke	Negative gene–smoking interaction effect	[196]
SULT1A1	Catalyzes transfer of the sulfonate group from the active sulfate to a substrate to form the respective sulfate or sulfamate ester	Negative gene–smoking interaction effects	[196]

(*continued*)

Table 10.2. (continued)

Gene	Functional role	Risk factor	References
UDP glycosyltransferases (UGTs) UGT1A7 variant	Catalyzes conjugation reactions where hydrophobic chemicals are transformed into water-soluble compounds. Potential maternal effects on embryonic development	Positive gene–smoking interaction effects	[41, 194, 196]
Developmental genes for oral clefts			
Transforming growth factor A (TGF α)	Transmembrane protein expressed at the medial edge of the epithelium (MEE) of fusing palatal shelves. Its receptor epidermal growth factor is expressed in the degenerating MEE	Positive gene–interaction effects (smoking, alcohol drinking, vitamins)	[85, 126]
Transforming growth factor β-3 (TGF β3)	Regulator of many biological processes such as proliferation, differentiation, epithelial-mesenchymal transformation, and apoptosis	Positive–gene interaction effects (smoking, alcohol drinking)	[126]
Muscle segment homeobox1 (MSX1)	Transcriptional repressor important in craniofacial, limb, and nervous system development	Positive–gene interaction effects (smoking and alcohol drinking)	[126, 138, 176]
MSX2	Similar to MSX1; rare cause of isolated cleft lip with or without cleft palate		[138, 176]
Acyl-CoA desaturase ACOD4	Pericentric inversion disrupts a gene (ACOD4) on chromosome 4q21 that codes for a novel acyl-CoA desaturase enzyme occurs in a single two generation family with CL		[15]
Retinoic acid receptor	Odds ratios for transmission of alleles at THRA1 were significant when ethnic group was included	Negative gene–smoking interaction effects	[126]
CHD7	Chromodomain helicase DNA-binding proteins		[53]
ESR1	Ligand-activated TF estrogen receptor		[154]
FGF/FGFR families FGF8 FGF3 FGF10 FGF18 FGFR1 FGFR2 FGFR3	Expressed during craniofacial development and can rarely harbor mutations that result in human clefting syndromes		[174]
SPRY1/SPRY2	Loss-of-function mutations in FGFR1 cause a syndromic form of clefting		[228]
TBX10	Ectopically expressed in dancer cleft lip and palate-mutant mice		[228]
GABRB3	β3 subunit of GABA receptor CL/P		[72]
GLI2	Mutations in GLi2 cause holoprosencephaly-like features with cleft lip and palate		[228]
ISGF3G	Similar to IRF6		[228]
Other candidate genes			
SKI, FOXE1, JAG2, LHX8	Rare causes of isolated cleft lip with or without cleft palate		[228]

at differential rates. The outermost layer of cuboidal cells is called the *external dental epithelium*, whereas the cells that encase the dental papilla are the *internal dental epithelium*. The latter gives rise to the ameloblasts, cells responsible for enamel formation. Functional layers develop within the enamel organ, which allow it to expand and change the fate of the dental papilla. The star-shaped cells of the stellate reticulum are rich in glycosaminoglycans that are thought to sequester fluids, as well as growth factors that lead to its expansion. The narrow layer of flattened cells termed the *stratum intermedium,* which expresses high levels of alkaline phosphatase, develops adjacent to the inner dental epithelium and influences differentiation into ameloblasts, as well as the process of enamel biomineralization.

Shortly after the shape of the crown is determined, the cells of the internal dental epithelium at the sites of the future cusp tips stop dividing and signal the underlying dental papilla cells to organize themselves along the epithelial–mesenchymal interface. These newly differentiated cells are called *odontoblasts*, cells that are responsible for the synthesis and secretion of

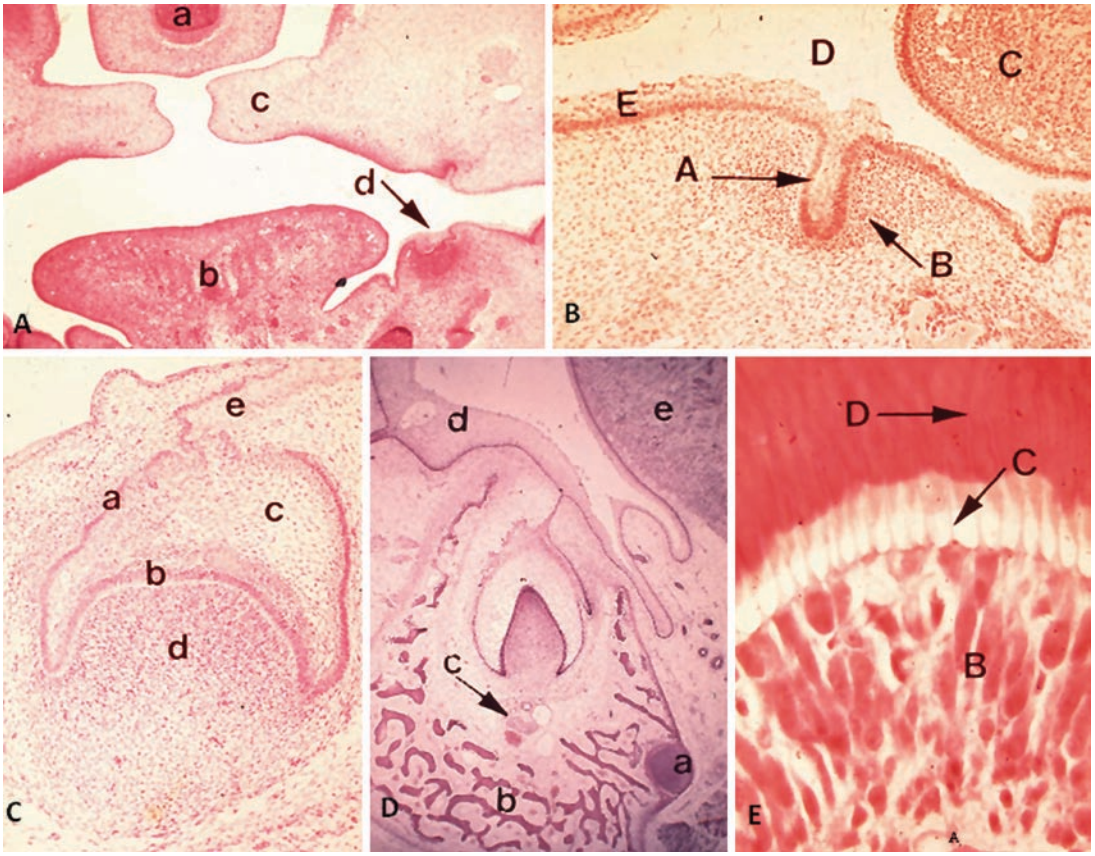

Figure 10.8. Histologic depiction of tooth development. (**a**) lamina phase (a = nasal septum; b = tongue; c = palatal shelves; d = dental lamina). (**b**) bud stage (a = ectodermal outgrowth; b = dental mesenchyme; c = tongue; d = oral cavity space; e = oral ectoderm). (**c**) cap or transition to early bell stage (a = outer dental epithelium; b = internal dental epithelium; c = stellate reticulum; d = dental papilla ectomesenchyme; e = dental lamina). (**d**) late bell stage (a = nerve bundle; b = alveolar bone; c = vasculature; d = oral ectoderm; e = tongue). Note the extension of the dental lamina on the right aspect of the dental organ that will form the succeeding incisor. (**e**) onset of dentinogenesis (a = dental pulp; b = cluster of odontoblasts that appear crowded at the tip; c = odontoblastic process; d = dentin).

the dentin matrix that shares the biochemical properties with bone. The dental papilla is now termed the dental *pulp*. After the odontoblasts deposit the first layer of predentin, inner dental epithelium cells develop into ameloblasts or enamel-producing cells. As enamel is deposited over the dentin matrix, the ameloblasts retreat to the outside and undergo apoptosis. In contrast, the odontoblasts line the inner surface of the dentin and remain metabolically active throughout the life of the tooth. The inner and outer dental epithelia combine in the area of the cervical loop to form Hertwig's epithelial root sheath. This structure influences the differentiation of the odontoblasts from the dental papilla, as well as the cementoblasts from the follicle mesenchyme, thus forming root dentin and cementum, respectively. The dental follicle that gives rise to the components of the periodontium (the periodontal ligament fibroblasts, alveolar bone of the tooth socket, and the cementum) also plays a role during tooth eruption, the last phase of odontogenesis.

10.6.1 Signaling Interactions During Tooth Development

Epithelial–mesenchymal interactions that involve an exchange of molecular information between the ectoderm and mesenchyme drive critical events in tooth morphogenesis. As is true for the

limb bud, no single transcription factor or growth factor is responsible for odontogenesis; rather, a series of interactions involving several molecules leads to the development of the complex tooth form [92]. Signaling is reciprocal in nature. In other words, information is exchanged to and from the dental epithelium to the mesenchyme, and to and from the dental mesenchyme to the epithelium. For example, when the dental epithelium is separated from the mesenchyme, cusp patterning does not occur. Similarly, in the absence of the dental epithelium, the dental mesenchyme does not differentiate into odontoblasts [177].

The website (http://honeybee.helsinki.fi/toothexp) lists all molecules expressed in tooth organs. The two principal groups of molecules involved in the information exchange between the tooth epithelium and mesenchyme are the transcription factors and growth factors. The key mediators are the BMPs, FGFs, and WNT families: *Shh*, transcriptional molecules like the *Msx-1,-2*homeoboxgenes,*l*ymphoid*e*nhancer-binding *f*actor *1* (Lef-1) and Pax9, a member of the paired-box-containing transcription factor gene family. For a description of the actions and interactions of these molecules, see [46, 100].

10.6.2 The Role of the Extracellular Matrix (ECM) in Tooth Morphogenesis and Cytodifferentiation

The basement membrane ECM directs the budding and folding of dental epithelium during morphogenesis [219]. Molecules like collagens type I, III, and IV, along with laminin and various proteoglycans, are expressed in the basement membrane at the tooth epithelial–mesenchymal interface [177]. The MMPs, such as gelatinase, cleave type-IV collagen and contribute to the overall degradation of the basement membrane [181]. The expression of the protease inhibitors, TIMPs 1, 2, and 3, also correlates with tooth morphogenesis. Earlier in vitro studies have shown that the integrity of the basement membrane influences the budding and folding of the dental epithelium during morphogenesis and the spatial ordering of the cells that undergo terminal differentiation [219].

10.6.3 Dentin Biomineralization

The formation of dentin follows the same principles that guide the formation of other mesenchymal-derived mineralized tissues, e.g., cementum or bone. The odontoblasts are terminally differentiated cells that synthesize and secrete a type-I collagen-rich organic matrix called *predentin*. As the odontoblasts retreat toward the center of the tooth, cellular processes that subsequently mineralize remain in the matrix. Apatite crystals are deposited in an orderly manner, with disruptions resulting in a defective matrix, as seen in several dentinogeneses imperfectae and dysplasias.

For several decades, researchers have sought answers to these fundamental questions:

What is the exact composition of the dentin matrix and what biochemical features distinguish dentin from bone and cementum? Are there dentin-specific markers and can they be used to characterize the nature of the replacement cells responsible for forming reparative dentin? How do the physical features and conformational structures of the ECM molecules facilitate the calcification of dentin? Do these macromolecules interact with each other during the mineralization of dentin, and do they form supramolecular complexes that promote the deposition of hydroxyapatite crystals? What is the nature of the ECM molecules that modulate the initiation, rate, and extent of dentin deposition? What is the nature of the genes that encode the ECM molecules for dentin? Are defects in these genes responsible for inherited dentin disorders, namely dentinogenesis imperfecta (DGI) and dentin dysplasia? What genes regulate the expression of key dentin ECM molecules?

The noncollagenous proteins (NCPs) of the dentin matrix are thought to play a unique role in modulating the mineralization of dentin. Experimental evidence for this role is provided by the discovery that mutations in the dentin sialophosphoprotein (DSPP) gene are responsible for the defects seen in DGI types II and III [51]. As 90% of the organic matrix is type-I collagen, it is not surprising that patients with DGI type I caused by mutations in the type-I collagen gene have severe dentinal defects [104].

In recent years, a family of dentin and bone NCPs that have attracted attention are the small

integrin-binding ligand, N-linked glycoprotein (SIBLING) proteins. This group comprises DSPP, dentin matrix protein 1 (DMP1), bone sialoprotein (BSP), and osteopontin (OPN) [57]. As they are polyanionic molecules, SIBLING proteins are active in the mineralization of a collagen-containing matrix and are also thought to promote crystal growth within predentin.

10.6.4 Dentin Sialophosphoprotein (DSPP)

DSPP is a member of the SIBLING family that makes dentin matrix distinguishable from the bone. It is transcribed from a single mRNA that encodes DSP at the 5′ end and DPP at the 3′ end of a large precursor molecule [175]. Because of the abundance of at least two proteins in dentin ECM, DSPP is likely to be cleaved posttranslationally by a proteinase. The expression profiles of DSP and DPP show tooth-specific colocalization in the preameloblasts, the newly differentiated odontoblasts in predentin and dentin matrix. DSPP does occur in bone, but with far fewer osteoblasts expressed than in dentin and odontoblasts [163].

DSP, discovered by Butler et al. [27], has no specific function to date; it does not promote mineralization in vitro [22]. In contrast, DPP, discovered by Veis and Perry [227], may modulate mineralization. It contains unusually large amounts of acidic residues, such as aspartic acid and phosphoserine, and can initiate and modulate the formation and growth of hydroxyapatite crystals [23]. Expressed in odontoblasts, DPP binds to collagen at the mineralization front and may modulate binding of other NCPs to the growing hydroxyapatite crystals in a specific conformation [118].

More recently, a proteoglycan form of DSP (DSP-PG) has been isolated and characterized, but its precise role is still unknown [210].

Each of the three DSPP-derived variants, DSP, DPP, and DSP-PG, may play a unique role in the mineralization of dentin. Conversion of DSPP to DSP and DPP in dentin may require activation and the activating molecule(s), once identified, may have therapeutic value. As the precursor of DSPP is a large molecule, it is likely that structural changes are needed to enable the DPP to bind calcium to the collagen fibers. In other words, the rate and extent of biomineralization may be controlled by the activation and structural changes involving DSPP.

Mutations in the human DSPP gene give rise to DGI-II and DGI-III, causing the dentin to be severely affected. Studies with DSPP-null mice have provided evidence for defects in the mineralization of dentin. The SIBLING protein, therefore, is critical for normal dentinogenesis [207].

10.7 Future Perspectives

Many medical and dental scientists envision the day when diagnostic tools will be available to establish a personalized genetic and molecular profile of a patient's condition. Such a "molecular fingerprint" can also provide clues about gene changes and their effects on encoded proteins and the signaling pathways that are altered in the development and progression of the disease. In coming decades, identification and analysis of molecules that regulate the development of the craniofacial complex will provide insights that can be translated into clinical therapies to treat the spectrum of disorders and diseases that affect this part of the body. Knowledge of the mechanisms that drive the formation of mandible, palate, TMJ, and the teeth will provide a basis for developing new regenerative strategies aimed at restoring normal structure and function.

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References

1. Abel EL (2006) Fetal alcohol syndrome: a cautionary note. Curr Pharm Des 12:1521–1529
2. Abidi FE, Miano MG, Murray JC, Schwartz CE (2007) A novel mutation in the PHF8 gene is associated with X-linked mental retardation with cleft lip/cleft palate. Clin Genet 72:19–22
3. Adab K, Sayne JR, Carlson DS, Opperman LA (2002) Elevated TGF-b1, TGF-b2, TGF-b3 and Msx2 expression are associated with morphogenesis of facial sutures and active bone growth from these sites. J Dent Res 81:A221
4. Adab K, Sayne JR, Carlson DS, Opperman LA (2003) Nasal capsular cartilage is required for rat transpalatal suture morphogenesis. Differentiation 71:496–505
5. Akita S, Hirano A (2005) Surgical modifications for microform cleft lip repairs. J Craniofac Surg 16: 1106–1110
6. Alappat SR, Zhang Z, Suzuki K, Zhang X, Liu H, Jiang R, Yamada G, Chen Y (2005) The cellular and molecular etiology of the cleft secondary palate in Fgf10 mutant mice. Dev Biol 277:102–113
7. Alkuraya FS, Saadi I, Lund JJ, Turbe-Doan A, Morton CC, Maas RL (2006) SUMO1 haploinsufficiency leads to cleft lip and palate. Science 313:1751
8. Almarza AJ, Athanasiou KA (2004) Design characteristics for the tissue engineering of cartilaginous tissues. Ann Biomed Eng 32:2–17
9. An S, Dickens MA, Bleu T, Hallmark OG, Goetzl EJ (1997) Molecular cloning of the human Edg2 protein and its identification as a functional cellular receptor for lysophosphatidic acid. Biochem Biophys Res Commun 231:619–622
10. Andreou AM, Pauws E, Jones MC, Singh MK, Bussen M, Doudney K, Moore GE, Kispert A, Brosens JJ, Stanier P (2007) TBX22 missense mutations found in patients with X-linked cleft palate affect DNA binding, sumoylation, and transcriptional repression. Am J Hum Genet 81:700–712
11. Avery JK (1987) Development, structure and function of temporomandibular joint, oral development and histology. Williams & Williams, Baltimore, pp 348–364
12. Barbosa AC, Funato N, Chapman S, McKee MD, Richardson JA, Olson EN, Yanagisawa H (2007) Hand transcription factors cooperatively regulate development of the distal midline mesenchyme. Dev Biol 310:154–168
13. Baume LJ (1962) Ontogenesis of the human temporomandibular joint 1. Development of the condyles. J Dent Res 41:1327–1338
14. Baume LJ, Holz J (1970) Ontogenesis of the human temporomandibular joint: 2. Development of the temporal components. J Dent Res 49:864–875
15. Beiraghi S, Zhou M, Talmadge CB, Went-Sumegi N, Davis JR, Huang D, Saal H, Seemayer TA, Sumegi J (2003) Identification and characterization of a novel gene disrupted by a pericentric inversion inv(4) (p13.1q21.1) in a family with cleft lip. Gene 309:11–21
16. Beresford WA (1981) Chondroid bone, secondary cartilage, and metaplasia. Urban and Schwartzenberg, Baltimore
17. Beverdam A, Merlo GR, Paleari L, Mantero S, Genova F, Barbieri O, Janvier P, Levi G (2002) Jaw transformation with gain of symmetry after Dlx5/Dlx6 inactivation: mirror of the past? Genesis 34: 221–227
18. Blanton SH, Kolle BS, Hecht JT, Mulliken JB, Martin ER (2000) No evidence supporting MTHFR as a risk factor in the development of familial NSCLP. Am J Med Genet 92:370–371
19. Blavier L, Lazaryev A, Groffen J, Heisterkamp N, DeClerck YA, Kaartinen V (2001) Tgf-beta3-induced palatogenesis requires matrix metalloproteinases. Mol Biol Cell 12:1457–1466
20. Boorman JG, Varma S, Ogilvie CM (2001) Velopharyngeal incompetence and chromosome 22q11 deletion. Lancet 357:774
21. Boot MJ, Steegers-Theunissen RP, Poelmann RE, Van Iperen L, Lindemans J, Gittenberger-de Groot AC (2003) Folic acid and homocysteine affect neural crest and neuroepithelial cell outgrowth and differentiation in vitro. Dev Dyn 227:301–308
22. Boskey A, Spevak L, Tan M, Doty SB, Butler WT (2000) Dentin sialoprotein (DSP) has limited effects on in vitro apatite formation and growth. Calcif Tissue Int 67:472–478
23. Boskey AL, Maresca M, Doty S, Sabsay B, Veis A (1990) Concentration-dependent effects of dentin phosphophoryn in the regulation of in vitro hydroxyapatite formation and growth. Bone Miner 11:55–65
24. Braybrook C, Doudney K, Marcano AC, Arnason A, Bjornsson A, Patton MA, Goodfellow PJ, Moore GE, Stanier P (2001) The T-box transcription factor gene TBX22 is mutated in X-linked cleft palate and ankyloglossia. Nat Genet 29:179–183
25. Briggs RM (1976) Vitamin supplementation as a possible factor in the incidence of cleft lip/palate deformities in humans. Clin Plast Surg 3:647–652
26. Britto JA, Evans RD, Hayward RD, Jones BM (2002) Toward pathogenesis of Apert cleft palate: FGF, FGFR, and TGF beta genes are differentially expressed in sequential stages of human palatal shelf fusion. Cleft Pal Craniofac J 39:332–340
27. Butler WT, Bhown M, Dimuzio MT, Linde A (1981) Nonocollagenous proteins of dentin. Isolation and partial characterization of rat dentin proteins and proteoglycans using a three-step preparative method. Coll Relat Res 1:187–199
28. Buxton PG, Hall B, Archer CW, Francis-West P (2003) Secondary chondrocyte-derived Ihh stimulates proliferation of periosteal cells during chick development. Development 130:4729–4739
29. Carette MJ, Ferguson MW (1992) The fate of medial edge epithelial cells during palatal fusion in vitro: an analysis by DiI labelling and confocal microscopy. Development 114:379–388
30. Carinci F, Pezzetti F, Scapoli L, Martinelli M, Carinci P, Tognon M (2000) Genetics of nonsyndromic cleft lip and palate: a review of international studies and data

regarding the Italian population. Cleft Pal Craniofac J 37:33–40
31. Carlson BM (2004) Human embryology and developmental biology, 3rd edn. Mosby, Philadelphia, vol xv, p 527
32. Carlson DS (1994) Growth of the temporomandibular joint. In: Zarb GA, Carlsson GE, Sessle BJ, Mohl ND (eds) Temporomandibular joint and masticatory muscle disorders. Munksgaard, Copenhagen 128–153
33. Castanet M, Park SM, Smith A, Bost M, Leger J, Lyonnet S, Pelet A, Czernichow P, Chatterjee K, Polak M (2002) A novel loss-of-function mutation in TTF-2 is associated with congenital hypothyroidism, thyroid agenesis and cleft palate. Hum Mol Genet 11:2051–2059
34. Cerny R, Lwigale P, Ericsson R, Meulemans D, Epperlein HH, Bronner-Fraser M (2004) Developmental origins and evolution of jaws: new interpretation of "maxillary" and "mandibular". Dev Biol 276:225–236
35. Chai Y, Maxson RE Jr (2006) Recent advances in craniofacial morphogenesis. Dev Dyn 235:2353–2375
36. Charite J, McFadden DG, Merlo G, Levi G, Clouthier DE, Yanagisawa M, Richardson JA, Olson EN (2001) Role of Dlx6 in regulation of an endothelin-1-dependent, dHAND branchial arch enhancer. Genes Dev 15: 3039–3049
37. Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, Westphal H, Beachy PA (1996) Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature 383:407–413
38. Christensen K, Juel K, Herskind AM, Murray JC (2004) Long term follow up study of survival associated with cleft lip and palate at birth. Br Med J 328:12
39. Clouthier DE, Hosoda K, Richardson JA, Williams SC, Yanagisawa H, Kuwaki T, Kumada M, Hammer RE, Yanagisawa M (1998) Cranial and cardiac neural crest defects in endothelin-A receptor-deficient mice. Development 125:813–824
40. Cole A, Lynch P, Slator R (2008) A new grading of Pierre Robin sequence. Cleft Pal Craniofac J 45:603–606
41. Collier AC, Tingle MD, Paxton JW, Mitchell MD, Keelan JA (2002) Metabolizing enzyme localization and activities in the first trimester human placenta: the effect of maternal and gestational age, smoking and alcohol consumption. Hum Reprod 17:2564–2572
42. Cowan CM, Cheng S, Ting K, Soo C, Walder B, Wu B, Kuroda S, Zhang X (2006) Nell-1 induced bone formation within the distracted intermaxillary suture. Bone 38:48–58
43. Creuzet S, Couly G, Le Douarin NM (2005) Patterning the neural crest derivatives during development of the vertebrate head: insights from avian studies. J Anat 207:447–459
44. Cuervo R, Covarrubias L (2004) Death is the major fate of medial edge epithelial cells and the cause of basal lamina degradation during palatogenesis. Development 131:15–24
45. Cuneo BF (2001) 22q11.2 deletion syndrome: DiGeorge, velocardiofacial, and conotruncal anomaly face syndromes. Curr Opin Pediatr 13:465–472
46. D'Souza RN, Klein OD (2007) Unraveling the molecular mechanisms that lead to supernumerary teeth in mice and men: current concepts and novel approaches. Cells Tissues Organs 186:60–69
47. Depew MJ, Lufkin T, Rubenstein JL (2002) Specification of jaw subdivisions by Dlx genes. Science 298:381–385
48. Depew MJ, Simpson CA, Morasso M, Rubenstein JL (2005) Reassessing the Dlx code: the genetic regulation of branchial arch skeletal pattern and development. J Anat 207:501–561
49. Dixon J, Jones NC, Sandell LL, Jayasinghe SM, Crane J, Rey JP, Dixon MJ, Trainor PA (2006) Tcof1/Treacle is required for neural crest cell formation and proliferation deficiencies that cause craniofacial abnormalities. Proc Natl Acad Sci U S A 103:13403–13408
50. Dode C, Fouveaut C, Mortier G, Janssens S, Bertherat J, Mahoudeau J, Kottler ML, Chabrolle C, Gancel A, Francois I, Devriendt K, Wolczynski S, Pugeat M, Pineiro-Garcia A, Murat A, Bouchard P, Young J, Delpech M, Hardelin JP (2007) Novel FGFR1 sequence variants in Kallmann syndrome, and genetic evidence that the FGFR1c isoform is required in olfactory bulb and palate morphogenesis. Hum Mutat 28:97–98
51. Dong J, Amor D, Aldred MJ, Gu T, Escamilla M, MacDougall M (2005) DLX3 mutation associated with autosomal dominant amelogenesis imperfecta with taurodontism. Am J Med Genet A 133A:138–141
52. Fang J, Dagenais SL, Erickson RP, Arlt MF, Glynn MW, Gorski JL, Seaver LH, Glover TW (2000) Mutations in FOXC2 (MFH-1), a forkhead family transcription factor, are responsible for the hereditary lymphedema-distichiasis syndrome. Am J Hum Genet 67:1382–1388
53. Felix TM, Hanshaw BC, Mueller R, Bitoun P, Murray JC (2006) CHD7 gene and non-syndromic cleft lip and palate. Am J Med Genet A 140:2110–2114
54. Ferguson MW (1988) Palate development. Development 103:41–60
55. Finnell RH, Shaw GM, Lammer EJ, Brandl KL, Carmichael SL, Rosenquist TH (2004) Gene-nutrient interactions: importance of folates and retinoids during early embryogenesis. [Review] [132 refs]. Toxicol Appl Pharmacol 198:75–85
56. Firulli AB, McFadden DG, Lin Q, Srivastava D, Olson EN (1998) Heart and extra-embryonic mesodermal defects in mouse embryos lacking the bHLH transcription factor Hand1. Nat Genet 18:266–270
57. Fisher LW, Torchia DA, Fohr B, Young MF, Fedarko NS (2001) Flexible structures of SIBLING proteins, bone sialoprotein, and osteopontin. Biochem Biophys Res Commun 280:460–465
58. Fitzpatrick DR, Denhez F, Kondaiah P, Akhurst RJ (1990) Differential expression of TGF beta isoforms in murine palatogenesis. Development 109:585–595
59. Fogh-Andersen P (1971) Epidemiology and etiology of clefts. In: Bergsma D (ed) Birth defects: original article series. Williams and Wilkins, Baltimore
60. Frank DU, Fotheringham LK, Brewer JA, Muglia LJ, Tristani-Firouzi M, Capecchi MR, Moon AM (2002) An

Fgf8 mouse mutant phenocopies human 22q11 deletion syndrome. Development 129:4591–4603
61. Fukada K, Shibata S, Ohya K, Kuroda T (1999) In situ hybridisation study of type I, II, X collagens and aggregan mRNSa in the developing condylar cartilage of fetal mouse mandible. J Anat 195:321–329
62. Funato N, Chapman SL, McKee MD, Funato H, Morris JA, Shelton JM, Richardson JA, Yanagisawa H (2009) Hand2 controls osteoblast differentiation in the branchial arch by inhibiting DNA binding of Runx2. Development 136:615–625
63. Furstman L (1963) The early development of the human temporomandibular joint. Am J Orthod 49:672–682
64. Galler KM, Cavender A, Yuwono V, Dong H, Shi S, Schmalz G, Hartgerink JD, D'Souza RN (2008) Self-assembling peptide amphiphile nanofibers as a scaffold for dental stem cells. Tissue Eng A 14:2051–2058
65. Gardner E, O'Rahilly R (1968) The early development of the knee joint in staged human embryos. J Anat 102:289–299
66. Gendron-Maguire M, Mallo M, Zhang M, Gridley T (1993) Hoxa-2 mutant mice exhibit homeotic transformation of skeletal elements derived from cranial neural crest. Cell 75:1317–1331
67. Gosain AK, Conley SF, Marks S, Larson DL (1996) Submucous cleft palate: diagnostic methods and outcomes of surgical treatment. Plast Reconstr Surg 97: 1497–1509
68. Grammatopoulos GA, Bell E, Toole L, Lumsden A, Tucker AS (2000) Homeotic transformation of branchial arch identity after Hoxa2 overexpression. Development 127:5355–5365
69. Green ML, Singh AV, Zhang Y, Nemeth KA, Sulik KK, Knudsen TB (2007) Reprogramming of genetic networks during initiation of the fetal alcohol syndrome. Dev Dyn 236:613–631
70. Griffith CM, Hay ED (1992) Epithelial-mesenchymal transformation during palatal fusion: carboxyfluorescein traces cells at light and electron microscopic levels. Development 116:1087–1099
71. Gritli-Linde A (2007) Molecular control of secondary palate development. Dev Biol 301:309–326
72. Gritli-Linde A (2008) The etiopathogenesis of cleft lip and cleft palate usefulness and caveats of mouse models. Curr Top Dev Biol 84:37–138
73. Habib H, Hatta T, Udagawa J, Zhang L, Yoshimura Y, Otani H (2005) Fetal jaw movement affects condylar cartilage development. J Dent Res 84:474–479
74. Hall BK (1968) The fate of adventitious and embryonic articular cartilage in the skull of the common fowl, Gallus Domesticus (Aves: Phasianidae). Aust J Zool 16:795–805
75. Hall BK (2005) Bones and cartilage: developmental and evolutionary skeletal biology. Elsevier, Amsterdam
76. Hanken J, Hall BK (eds) (1993) The skull, vol 1. Development, University of Chicago Press, Chicago, XIV, p 587 S
77. Hanken J, Hall BK (eds) (1993) The skull, volume 3: Functional and evolutionary mechanisms. University of Chicago Press, Chicago, X, p 460 S
78. Hardingham TE, Oldershaw RA, Tew SR (2006) Cartilage, SOX9 and Notch signals in chondrogenesis. J Anat 209:469–480
79. Hay ED (1995) An overview of epithelio-mesenchymal transformation. Acta Anat 154:8–20
80. Hilliard SA, Yu L, Gu S, Zhang Z, Chen YP (2005) Regional regulation of palatal growth and patterning along the anterior-posterior axis in mice. J Anat 207:655–667
81. Hines RN, McCarver DG (2002) The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes. J Pharmacol Exp Ther 300:355–360
82. Hinton RJ (1988) Response of the intermaxillary suture cartilage to alterations in masticatory function. Anat Rec 220:376–387
83. Hou B, Fukai N, Olsen BR (2007) Mechanical force-induced midpalatal suture remodeling in mice. Bone 40:1483–1493
84. Humphrey T (1968) The development of mouth opening and related rflexes involving the oral area of human fetuses. Ala J Med Sci 5:126–157
85. Hwang SJ, Beaty TH, Panny SR, Street NA, Joseph JM, Gordon S, McIntosh I, Francomano CA (1995) Association study of transforming growth factor alpha (TGF alpha) TaqI polymorphism and oral clefts: indication of gene-environment interaction in a population-based sample of infants with birth defects. Am J Epidemiol 141:629–636
86. Ingervall B, Carlsson GE, Thilander B (1976) Postnatal development of the human temporomandibular joint. I. A microradiographic study. Acta Odontol Scand 34:133–139
87. Ishii M, Han J, Yen HY, Sucov HM, Chai Y, Maxson RE Jr (2005) Combined deficiencies of Msx1 and Msx2 cause impaired patterning and survival of the cranial neural crest. Development 132:4937–4950
88. Itikala PR, Watkins ML, Mulinare J, Moore CA, Liu Y (2001) Maternal multivitamin use and orofacial clefts in offspring. Teratology 63:79–86
89. Iwasaki M, Le AX, Helms JA (1997) Expression of indian hedgehog, bone morphogenetic protein 6 and gli during skeletal morphogenesis. Mech Dev 69:197–202
90. Jeong J, Mao J, Tenzen T, Kottmann AH, McMahon AP (2004) Hedgehog signaling in the neural crest cells regulates the patterning and growth of facial primordia. Genes Dev 18:937–951
91. Jernvall J, Kettunen P, Karavanova I, Martin LB, Thesleff I (1994) Evidence for the role of the enamel knot as a control center in mammalian tooth cusp formation: non-dividing cells express growth stimulating Fgf-4 gene. Int J Dev Biol 38:463–469
92. Jernvall J, Thesleff I (2000) Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech Dev 92:19–29
93. Jiang R, Bush JO, Lidral AC (2006) Development of the upper lip: morphogenetic and molecular mechanisms. Dev Dyn 235:spc1
94. Jin J-Z, Ding J (2006) Analysis of cell migration, transdifferentiation and apoptosis during mouse secondary palate fusion. Development 133:3341–3347

95. Kaartinen V, Voncken JW, Shuler C, Warburton D, Bu D, Heisterkamp N, Groffen J (1995) Abnormal lung development and cleft palate in mice lacking TGF-beta 3 indicates defects of epithelial-mesenchymal interaction. Nat Genet 11:415–421
96. Kaartinen V, Cui XM, Heisterkamp N, Groffen J, Shuler CF (1997) Transforming growth factor-beta3 regulates transdifferentiation of medial edge epithelium during palatal fusion and associated degradation of the basement membrane. Dev Dyn 209:255–260
97. Kaartinen V, Haataja L, Nagy A, Heisterkamp N, Groffen J (2002) TGFbeta3-induced activation of RhoA/Rho-kinase pathway is necessary but not sufficient for epithelio-mesenchymal transdifferentiation: implications for palatogenesis. Int J Mol Med 9:563–570
98. Kallen B, Mastroiacovo P, Robert E (1996) Major congenital malformations in Down syndrome. Am J Med Genet 65:160–166
99. Kang P, Svoboda KK (2005) Epithelial-mesenchymal transformation during craniofacial development. J Dent Res 84:678–690
100. Kapadia H, Mues G, D'Souza R (2007) Genes affecting tooth morphogenesis. Orthod Craniofac Res 10:237–244
101. Karsenty G (2008) Transcriptional control of skeletogenesis. Annu Rev Genomics Hum Genet 9:183–196
102. Keith DA (1982) Development of the human temporomandibular joint. Br J Oral Surg 20:217–224
103. Kerrigan JJ, Mansell JP, Sengupta A, Brown N, Sandy JR (2000) Palatogenesis and potential mechanisms for clefting. J R Coll Surg Edinb 45:351–358
104. Kim JW, Simmer JP (2007) Hereditary dentin defects. J Dent Res 86:392–399
105. Kjaer I (1992) Human prenatal palatal shelf elevation related to craniofacial skeletal maturation. Eur J Orthod 14:26–30
106. Kjaer I (1997) Mandibular movements during elevation and fusion of palatal shelves evaluated from the course of Meckel's cartilage. J Craniofac Genet Dev Biol 17:80–85
107. Kobayashi ET, Hashimoto F, Kobayashi Y, Sakai E, Miyazaki Y, Kamiya T, Kobayashi K, Kato Y, Sakai H (1999) Force-induced rapid changes in cell fate at midpalatal suture cartilage of growing rats. J Dent Res 78:1495–1504
108. Kokavec R (2006) Goldenhar syndrome with various clinical manifestations. Cleft Palate Craniofac J 43: 628–634
109. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T (1997) Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 89:755–764
110. Komori T (2006) Regulation of osteoblast differentiation by transcription factors. J Cell Biochem 99:1233–1239
111. Kondo S, Schutte BC, Richardson RJ, Bjork BC, Knight AS, Watanabe Y, Howard E, de Lima RL, Daack-Hirsch S, Sander A, McDonald-McGinn DM, Zackai EH, Lammer EJ, Aylsworth AS, Ardinger HH, Lidral AC, Pober BR, Moreno L, Arcos-Burgos M, Valencia C, Houdayer C, Bahuau M, Moretti-Ferreira D, Richieri-Costa A, Dixon MJ, Murray JC (2002) Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. Nat Genet 32: 285–289
112. Lammer EJ, Shaw GM, Iovannisci DM, Finnell RH (2004) Periconceptional multivitamin intake during early pregnancy, genetic variation of acetyl-N-transferase 1 (NAT1), and risk for orofacial clefts. Birth Defects Res A Clin Mol Teratol 70:846–852
113. Lammer EJ, Shaw GM, Iovannisci DM, Van Waes J, Finnell RH (2004) Maternal smoking and the risk of orofacial clefts: Susceptibility with NAT1 and NAT2 polymorphisms. Epidemiology 15:150–156
114. Lanctot C, Moreau A, Chamberland M, Tremblay ML, Drouin J (1999) Hindlimb patterning and mandible development require the Ptx1 gene. Development 126:1805–1810
115. Le Douarin NM, Brito JM, Creuzet S (2007) Role of the neural crest in face and brain development. Brain Res Rev 55:237–247
116. Lee SH, Bedard O, Buchtova M, Fu K, Richman JM (2004) A new origin for the maxillary jaw. Dev Biol 276:207–224
117. Lee SK, Kim YS, Oh HS, Yang KH, Kim EC, Chi JG (2001) Prenatal development of the human mandible. Anat Rec 263:314–325
118. Linde A, Goldberg M (1993) Dentinogenesis. Crit Rev Oral Biol Med 4:679–728
119. Liu C, Song R, Song Y (2000) Sutural expansion osteogenesis for management of the bony-tissue defect in cleft palate repair: experimental studies in dogs. Plast Reconstr Surg 105:2012–2025; discussion 26–27
120. Liu W, Selever J, Murali D, Sun X, Brugger SM, Ma L, Schwartz RJ, Maxson R, Furuta Y, Martin JF (2005) Threshold-specific requirements for Bmp4 in mandibular development. Dev Biol 283:282–293
121. Lohnes D, Mark M, Mendelsohn C, Dolle P, Dierich A, Gorry P, Gansmuller A, Chambon P (1994) Function of the retinoic acid receptors (RARs) during development (I). Craniofacial and skeletal abnormalities in RAR double mutants. Development 120: 2723–2748
122. Lohnes D, Mark M, Mendelsohn C, Dolle P, Decimo D, LeMeur M, Dierich A, Gorry P, Chambon P (1995) Developmental roles of the retinoic acid receptors. J Steroid Biochem Mol Biol 53:475–486
123. Luder HU (1997) Frequency and distribution of articular tissue features in adult human mandibular condyles: a semiquantitative light microscopic study. Anat Rec 248:18–28
124. Luder HU (1998) Age changes in the articular tissue of human mandibular condyles from adolescence to old age: a semiquantitative light microscopic study. Anat Rec 251:439–447
125. Mackie EJ, Ahmed YA, Tatarczuch L, Chen KS, Mirams M (2008) Endochondral ossification: how cartilage is converted into bone in the developing skeleton. Int J Biochem Cell Biol 40:46–62
126. Maestri NE, Beaty TH, Hetmanski J, Smith EA, McIntosh I, Wyszynski DF, Liang KY, Duffy DL, VanderKolk C (1997) Application of transmission disequilibrium tests to nonsyndromic oral clefts: including candidate

genes and environmental exposures in the models. Am J Med Genet 73:337–344
127. Marazita ML (2007) Subclinical features in non-syndromic cleft lip with or without cleft palate (CL/P): review of the evidence that subepithelial orbicularis oris muscle defects are part of an expanded phenotype for CL/P. Orthod Craniofac Res 10:82–87
128. Marcano AC, Doudney K, Braybrook C, Squires R, Patton MA, Lees MM, Richieri-Costa A, Lidral AC, Murray JC, Moore GE, Stanier P (2004) TBX22 mutations are a frequent cause of cleft palate. J Med Gen 41:68–74
129. Mark M, Lohnes D, Mendelsohn C, Dupe V, Vonesch JL, Kastner P, Rijli F, Bloch-Zupan A, Chambon P (1995) Roles of retinoic acid receptors and of Hox genes in the patterning of the teeth and of the jaw skeleton. Int J Dev Biol 39:111–121
130. Martelli H Jr, Paranaiba LM, de Miranda RT, Orsi J Jr, Coletta RD (2008) Apert syndrome: report of a case with emphasis on craniofacial and genetic features. Pediatr Dent 30:464–468
131. Marti E, Bovolenta P (2002) Sonic hedgehog in CNS development: one signal, multiple outputs. Trends Neurosci 25:89–96
132. Martinelli M, Scapoli L, Pezzetti F, Carinci F, Carinci P, Stabellini G, Bisceglia L, Gombos F, Tognon M (2001) C677T variant form at the MTHFR gene and CL/P: a risk factor for mothers? Am J Med Genet 98:357–360
133. Martinez-Alvarez C, Blanco MJ, Perez R, Rabadan MA, Aparicio M, Resel E, Martinez T, Nieto MA (2004) Snail family members and cell survival in physiological and pathological cleft palates. Dev Biol 265:207–218
134. Mendelsohn C, Lohnes D, Decimo D, Lufkin T, LeMeur M, Chambon P, Mark M (1994) Function of the retinoic acid receptors (RARs) during development (II). Multiple abnormalities at various stages of organogenesis in RAR double mutants. Development 120:2749–2771
135. Merida-Velasco JR, Rodriguez-Vasquez JF, Merida-Velasco JA, Sanchez-Montesinos I, Espin-Ferra J, Jimenez-Collado J (1999) Development of the human temporomandibular joint. Anat Rec 255:20–33
136. Miettinen PJ, Chin JR, Shum L, Slavkin HC, Shuler CF, Derynck R, Werb Z (1999) Epidermal growth factor receptor function is necessary for normal craniofacial development and palate closure. Nat Genet 22:69–73
137. Milunsky JM, Maher TA, Zhao G, Roberts AE, Stalker HJ, Zori RT, Burch MN, Clemens M, Mulliken JB, Smith R, Lin AE (2008) TFAP2A mutations result in branchio-oculo-facial syndrome. Am J Hum Genet 82:1171–1177
138. Mitchell LE, Murray JC, O'Brien S, Christensen K (2001) Evaluation of two putative susceptibility loci for oral clefts in the Danish population. Am J Epidemiol 153:1007–1015
139. Moffett BC, DeHaan RL, Ursprung H (1965) The morphogenesis of joints, organogenesis. Holt, Rinehart, and Winston, New York, pp 301–313
140. Moreno F, Zuazo E, Gonzalez S, Bereciartu P (2009) 22q11 deletion syndrome: an expanding phenotype. Neurologia 24:69–71
141. Mori C, Nakamura N, Okamoto Y, Osawa M, Shiota K (1994) Cytochemical identification of programmed cell death in the fusing fetal mouse palate by specific labelling of DNA fragmentation. Anat Embryol (Berl) 190:21–28
142. Muenke M (2002) The pit, the cleft and the web. Nat Genet 32:219–220
143. Murray JC (2002) Gene/environment causes of cleft lip and/or palate. Clin Genet 61:248–256
144. Murray JC, Schutte BC (2004) Cleft palate: players, pathways, and pursuits. J Clin Invest 113: 1676–1678
145. Nawshad A, Hay ED (2003) TGFbeta3 signaling activates transcription of the LEF1 gene to induce epithelial mesenchymal transformation during mouse palate development. J Cell Biol 163:1291–1301
146. Nawshad A, LaGamba D, Hay ED (2004) Transforming growth factor beta (TGFbeta) signalling in palatal growth, apoptosis and epithelial mesenchymal transformation (EMT). Arch Oral Biol 49: 675–689
147. Nawshad A (2008) Palatal seam disintegration: To die or not to die? that is no longer the question. Dev Dyn 237:2643–2656
148. Neiswanger K, Weinberg SM, Rogers CR, Brandon CA, Cooper ME, Bardi KM, Deleyiannis FW, Resick JM, Bowen A, Mooney MP, de Salamanca JE, Gonzalez B, Maher BS, Martin RA, Marazita ML (2007) Orbicularis oris muscle defects as an expanded phenotypic feature in nonsyndromic cleft lip with or without cleft palate. Am J Med Genet A 143: 1143–1149
149. Nie X, Luukko K, Kettunen P (2006) FGF signalling in craniofacial development and developmental disorders. Oral Dis 12:102–111
150. Noden DM, Trainor PA (2005) Relations and interactions between cranial mesoderm and neural crest populations. J Anat 207:575–601
151. Ogutcen-Toller M, Juniper RP (1993) The embryonic development of the human lateral pterygoid muscle and its relationships with the temporomandibular joint disc and Meckel's cartilage. J Oral Maxillofac Surg 51:772–778
152. Okano J, Suzuki S, Shiota K (2006) Regional heterogeneity in the developing palate: morphological and molecular evidence for normal and abnormal palatogenesis. Congenit Anom (Kyoto) 46:49–54
153. Opperman LA (2000) Cranial sutures as intramembranous bone growth sites. Dev Dyn 219:472–485
154. Osoegawa K, Vessere GM, Utami KH, Mansilla MA, Johnson MK, Riley BM, L'Heureux J, Pfundt R, Staaf J, van der Vliet WA, Lidral AC, Schoenmakers EF, Borg A, Schutte BC, Lammer EJ, Murray JC, de Jong PJ (2008) Identification of novel candidate genes associated with cleft lip and palate using array comparative genomic hybridisation. J Med Genet 45:81–86
155. Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, Stamp GW, Beddington RS, Mundlos S, Olsen BR, Selby PB, Owen MJ (1997) Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell 89:765–771

156. Pasqualetti M, Ori M, Nardi I, Rijli FM (2000) Ectopic Hoxa2 induction after neural crest migration results in homeosis of jaw elements in Xenopus. Development 127:5367–5378
157. Pauws E, Stanier P (2007) FGF signalling and SUMO modification: new players in the aetiology of cleft lip and/or palate. Trends Genet 23:631–640
158. Pelton RW, Hogan BL, Miller DA, Moses HL (1990) Differential expression of genes encoding TGFs beta 1, beta 2, and beta 3 during murine palate formation. Dev Biol 141:456–460
159. Perry HT, Xu Y, Forbes DP (1985) The embryology of the temporomandibular joint. J Craniomand Prac 3:125–132
160. Petrovic A (1972) Mechanisms and regulation of mandibular condylar growth. Acta Morphol Neerl Scand 10:25–34
161. Petrovic AG, Stutzmann JJ, Oudet CL, McNamara JA Jr (1975) Control processes in the postnatal growth of the condylar cartilage of the mandible, determinants of mandibular form and growth. Center for Human Growth and Development, University of Michigan, Ann Arbor, pp 101–154
162. Prows CA, Bender PL (1999) Beyond Pierre Robin sequence. Neonatal Netw 18:13–19
163. Qin C, Brunn JC, Cadena E, Ridall A, Tsujigiwa H, Nagatsuka H, Nagai N, Butler WT (2002) The expression of dentin sialophosphoprotein gene in bone. J Dent Res 81:392–394
164. Qiu M, Bulfone A, Martinez S, Meneses JJ, Shimamura K, Pedersen RA, Rubenstein JL (1995) Null mutation of Dlx-2 results in abnormal morphogenesis of proximal first and second branchial arch derivatives and abnormal differentiation in the forebrain. Genes Dev 9:2523–2538
165. Qiu M, Bulfone A, Ghattas I, Meneses JJ, Christensen L, Sharpe PT, Presley R, Pedersen RA, Rubenstein JL (1997) Role of the Dlx homeobox genes in proximodistal patterning of the branchial arches: mutations of Dlx-1, Dlx-2, and Dlx-1 and -2 alter morphogenesis of proximal skeletal and soft tissue structures derived from the first and second arches. Dev Biol 185:165–184
166. Rabie ABM, She TT, Hagg U (2003) Functional appliance therapy accelerates and enhances condylar growth. Am J Orthod Dentofac Orthop 123:40–48
167. Radlanski RJ, Lieck S, Bontschev NE (1999) Development of the human temporomandibular joint. Computer-aided 3D-reconstructions. Eur J Oral Sci 107:25–34
168. Radlanski RJ, Renz H, Klarkowski MC (2003) Prenatal development of the human mandible. 3D reconstructions, morphometry and bone remodelling pattern, sizes 12–117 mm CRL. Anat Embryol (Berl) 207:221–232
169. Radlanski RJ, Renz H (2006) Genes, forces, and forms: mechanical aspects of prenatal craniofacial development. Dev Dyn 235:1219–1229
170. Rice R, Spencer-Dene B, Connor EC, Gritli-Linde A, McMahon AP, Dickson C, Thesleff I, Rice DP (2004) Disruption of Fgf10/Fgfr2b-coordinated epithelial-mesenchymal interactions causes cleft palate. J Clin Invest 113:1692–1700
171. Rice R, Thesleff I, Rice DPC (2005) Regulation of Twist, Snail, and Id1 is conserved between the developing murine palate and tooth. Dev Dyn 234:28–35
172. Richman JM, Buchtova M, Boughner JC (2006) Comparative ontogeny and phylogeny of the upper jaw skeleton in amniotes. Dev Dyn 235:1230–1243
173. Rijli FM, Mark M, Lakkaraju S, Dierich A, Dolle P, Chambon P (1993) A homeotic transformation is generated in the rostral branchial region of the head by disruption of Hoxa-2, which acts as a selector gene. Cell 75:1333–1349
174. Riley BM, Mansilla MA, Ma J, Daack-Hirsch S, Maher BS, Raffensperger LM, Russo ET, Vieira AR, Dode C, Mohammadi M, Marazita ML, Murray JC (2007) Impaired FGF signaling contributes to cleft lip and palate. Proc Natl Acad Sci U S A 104:4512–4517
175. Ritchie HH, Li X (2001) A novel rat dentin mRNA coding only for dentin sialoprotein. Eur J Oral Sci 109:342–347
176. Romitti PA, Lidral AC, Munger RG, Daack-Hirsch S, Burns TL, Murray JC (1999) Candidate genes for nonsyndromic cleft lip and palate and maternal cigarette smoking and alcohol consumption: evaluation of genotype-environment interactions from a population-based case-control study of orofacial clefts. Teratology 59:39–50
177. Ruch JV (1987) Determinisms of odontogenesis. Revis Biol Celular 14:1–99
178. Ruest LB, Dager M, Yanagisawa H, Charite J, Hammer RE, Olson EN, Yanagisawa M, Clouthier DE (2003) dHAND-Cre transgenic mice reveal specific potential functions of dHAND during craniofacial development. Dev Biol 257:263–277
179. Ruest LB, Xiang X, Lim KC, Levi G, Clouthier DE (2004) Endothelin-A receptor-dependent and -independent signaling pathways in establishing mandibular identity. Development 131:4413–4423
180. Ruest LB, Clouthier DE (2009) Elucidating timing and function of endothelin-A receptor signaling during craniofacial development using neural crest cell-specific gene deletion and receptor antagonism. Dev Biol 328:94–108
181. Sahlberg C, Reponen P, Tryggvason K, Thesleff I (1992) Association between the expression of murine 72 kDa type IV collagenase by odontoblasts and basement membrane degradation during mouse tooth development. Arch Oral Biol 37:1021–1030
182. Sanlaville D, Etchevers HC, Gonzales M, Martinovic J, Clement-Ziza M, Delezoide AL, Aubry MC, Pelet A, Chemouny S, Cruaud C, Audollent S, Esculpavit C, Goudefroye G, Ozilou C, Fredouille C, Joye N, Morichon-Delvallez N, Dumez Y, Weissenbach J, Munnich A, Amiel J, Encha-Razavi F, Lyonnet S, Vekemans M, Attie-Bitach T (2006) Phenotypic spectrum of CHARGE syndrome in fetuses with CHD7 truncating mutations correlates with expression during human development. J Med Genet 43:211–217
183. Sano K, Sekine J, Pe MB, Inokuchi T (1992) Bromodeoxyuridine Immunohistochemistry for Evaluating Age-Related Changes in the Rat Mandibular Condyle Decalcified by Intravenous Infusion. Biotech Histochem 67(5):297–302

184. Santagati F, Rijli FM (2003) Cranial neural crest and the building of the vertebrate head. Nat Rev Neurosci 4:806–818
185. Sato T, Kawamura Y, Asai R, Amano T, Uchijima Y, Dettlaff-Swiercz DA, Offermanns S, Kurihara Y, Kurihara H (2008) Recombinase-mediated cassette exchange reveals the selective use of Gq/G11-dependent and -independent endothelin 1/endothelin type A receptor signaling in pharyngeal arch development. Development 135:755–765
186. Sato T, Kurihara Y, Asai R, Kawamura Y, Tonami K, Uchijima Y, Heude E, Ekker M, Levi G, Kurihara H (2008) An endothelin-1 switch specifies maxillomandibular identity. Proc Natl Acad Sci U S A 105:18806–18811
187. Satokata I, Maas R (1994) Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. Nat Genet 6:348–356
188. Satokata I, Ma L, Ohshima H, Bei M, Woo I, Nishizawa K, Maeda T, Takano Y, Uchiyama M, Heaney S, Peters H, Tang Z, Maxson R, Maas R (2000) Msx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation. Nat Genet 24:391–395
189. Sawada M, Shimizu N (1996) Stimulation of bone formation in the expanding mid-palatal suture by transforming growth factor-beta 1 in the rat. Eur J Orthod 18:169–179
190. Schneider RA, Helms JA (2003) The cellular and molecular origins of beak morphology. Science 299:565–568
191. Schock F, Perrimon N (2002) Molecular mechanisms of epithelial morphogenesis. Annu Rev Cell Dev Biol 18:463–493
192. Shaw GM, Lammer EJ, Zhu H, Baker MW, Neri E, Finnell RH (2002) Maternal periconceptional vitamin use, genetic variation of infant reduced folate carrier (A80G), and risk of spina bifida.. Am J Med Genet 108:1–6. Erratum (2002) 113(4):392
193. Shaw GM, Zhu H, Lammer EJ, Yang W, Finnell RH (2003) Genetic variation of infant reduced folate carrier (A80G) and risk of orofacial and conotruncal heart defects. Am J Epidemiol 158:747–752
194. Shaw WC, Brattstrom V, Molsted K, Prahl-Andersen B, Roberts CT, Semb G (2005) The Eurocleft study: intercenter study of treatment outcome in patients with complete cleft lip and palate. Part 5: discussion and conclusions. Cleft Pal Craniofac J 42:93–98
195. Sherer DM, Metlay LA, Woods JR Jr (1995) Lack of mandibular movement manifested by absent fetal swallowing: a possible factor in the pathogenesis of micrognathia. Am J Perinat 12:30–33
196. Shi M, Wehby GL, Murray JC (2008) Review on genetic variants and maternal smoking in the etiology of oral clefts and other birth defects. Birth Defects Res C Embryo Today 84:16–29
197. Shi S, Gronthos S (2003) Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J Bone Miner Res 18:696–704
198. Shibata S, Suzuki S, Tengan T, Ishi M, Kuroda T (1996) A histological study of the developing condylar cartilage of the fetal mouse mandible using coronal sections. Arch Oral Biol 41:47–54
199. Shibata S, Fukada K, Suzuki S, Yamashita Y (1997) Immunohistochemistry of collagen types II and X, and enzyme-histochemistry of alkaline phosphatase in developing condylar cartilage of the fetal mouse mandible. J Anat 191:561–570
200. Shibata S, Suda N, Yoda S, Fukuoka H, Ohyama K, Yamashita Y, Komori T (2004) Runx2-deficient mice lack manmdibular condylar cartilage and have deformed Meckel's cartilage. Anat Embryol 208:273–280
201. Shibata S, Suda N, Suzuki S, Fukuoka H, Yamashita Y (2006) An in situ hybridization study of Runx2, Osterix, and Sox9 at the onset of condylar cartilage formation in fetal mouse mandible. J Anat 208: 169–177
202. Shuler CF, Halpern DE, Guo Y, Sank AC (1992) Medial edge epithelium fate traced by cell lineage analysis during epithelial-mesenchymal transformation in vivo. Dev Biol 154:318–330
203. Sim E, Payton M, Noble M, Minchin R (2000) An update on genetic, structural and functional studies of arylamine N-acetyltransferases in eucaryotes and procaryotes. Hum Mol Genet 9:2435–2441
204. Slavkin HC (1992) Incidence of cleft lips, palates rising. J Am Dent Assoc 123:61–65
205. Smelt VA, Upton A, Adjaye J, Payton MA, Boukouvala S, Johnson N, Mardon HJ, Sim E (2000) Expression of arylamine N-acetyltransferases in pre-term placentas and in human pre-implantation embryos. Hum Mol Genet 9:1101–1107
206. Snead MP, Yates JR (1999) Clinical and Molecular genetics of Stickler syndrome. J Med Genet 36:353–359
207. Sreenath T, Thyagarajan T, Hall B, Longenecker G, D'Souza R, Hong S, Wright JT, MacDougall M, Sauk J, Kulkarni AB (2003) Dentin sialophosphoprotein knockout mouse teeth display widened predentin zone and develop defective dentin mineralization similar to human dentinogenesis imperfecta type III. J Biol Chem 278:24874–24880
208. Srivastava D, Thomas T, Lin Q, Kirby ML, Brown D, Olson EN (1997) Regulation of cardiac mesodermal and neural crest development by the bHLH transcription factor, dHAND. Nat Genet 16:154–160
209. St Amand TR, Zhang Y, Semina EV, Zhao X, Hu Y, Nguyen L, Murray JC, Chen Y (2000) Antagonistic signals between BMP4 and FGF8 define the expression of Pitx1 and Pitx2 in mouse tooth-forming anlage. Dev Biol 217:323–332
210. Sugars RV, Olsson ML, Waddington R, Wendel M (2006) Substitution of bovine dentine sialoprotein with chondroitin sulfate glycosaminoglycan chains. Eur J Oral Sci 114:89–92
211. Sun D, Baur S, Hay ED (2000) Epithelial-mesenchymal transformation is the mechanism for fusion of the craniofacial primordia involved in morphogenesis of the chicken lip. Dev Biol 228: 337–349
212. Suzuki K, Hu D, Bustos T, Zlotogora J, Richieri-Costa A, Helms JA, Spritz RA (2000) Mutations of PVRL1, encoding a cell-cell adhesion molecule/herpesvirus receptor, in cleft lip/palate-ectodermal dysplasia. Nat Genet 25:427–430
213. Sweeney L (1998) Basic concepts in embryology: a student's survival guide. McGraw-Hill, New Jersy, p 443

214. Takahashi I, Mizoguchi I, Nakamura M, Sasano Y, Saitoh S, Kagayama M, Mitani H (1996) Effects of expansive force on the differentiation of midpalatal suture cartilage in rats. Bone 18:341–348
215. Takenoshita Y (1982) Development with age of the human mandibular condyle: part II. Assessment of mineral content and microradiographic study. Dentomaxillofac Radiol 11:17–24
216. Tang LS, Santillano DR, Wlodarczyk BJ, Miranda RC, Finnell RH (2005) Role of Folbp1 in the regional regulation of apoptosis and cell proliferation in the developing neural tube and craniofacies. Am J Med Genet C Semin Med Genet 135:48–58
217. Taniguchi K, Sato N, Uchiyama Y (1995) Apoptosis and heterophagy of medial edge epithelial cells of the secondary palatine shelves during fusion. Arch Histol Cytol 58:191–203
218. Ten Cate A (1998) Oral histology: development, structure, and function, 5th edn, Mosby, St. Louis.
219. Thesleff I, Pratt RM (1980) Tunicamycin inhibits mouse tooth morphogenesis and odontoblast differentiation in vitro. J Embryol Exp Morphol 58:195–208
220. Tomo S, Ogita M, Tomo I (1997) Development of mandibular cartilages in the rat. Anat Rec 249: 233–239
221. Trainor P, Krumlauf R (2000) Plasticity in mouse neural crest cells reveals a new patterning role for cranial mesoderm. Nat Cell Biol 2:96–102
222. Trotman CA, Hou D, Burdi AR, Cohen SR, Carlson DS (1995) Histomorphologic analysis of the soft palate musculature in prenatal cleft and noncleft A/Jax mice. Cleft Pal Craniofac J 32:455–462
223. Trumpp A, Depew MJ, Rubenstein JL, Bishop JM, Martin GR (1999) Cre-mediated gene inactivation demonstrates that FGF8 is required for cell survival and patterning of the first branchial arch. Genes Dev 13:3136–3148
224. Tucker AS, Al Khamis A, Ferguson CA, Bach I, Rosenfeld MG, Sharpe PT (1999) Conserved regulation of mesenchymal gene expression by Fgf-8 in face and limb development. Development 126: 221–228
225. Tucker AS, Yamada G, Grigoriou M, Pachnis V, Sharpe PT (1999) Fgf-8 determines rostral-caudal polarity in the first branchial arch. Development 126:51–61
226. van der Linden EJ, Burdi AR, de Jongh HJ (1987) Critical periods in the prenatal morphogenesis of the human lateral pterygoid muscle, the mandibular condyle, the articular disc, and medial articular capsule. Am J Orthod 91:22–28
227. Veis A, Perry A (1967) The phosphoprotein of the dentin matrix. Biochem 6:2409–2416
228. Vieira AR, Avila JR, Daack-Hirsch S, Dragan E, Felix TM, Rahimov F, Harrington J, Schultz RR, Watanabe Y, Johnson M, Fang J, O'Brien SE, Orioli IM, Castilla EE, Fitzpatrick DR, Jiang R, Marazita ML, Murray JC (2005) Medical sequencing of candidate genes for nonsyndromic cleft lip and palate. PLoS Genet 1:e64
229. Vij K, Mao JJ (2006) Geometry and cell density of rat craniofacial sutures during early postnatal development and upon in vivo cyclic loading. Bone 38:722–730
230. Vilkki SK, Hukki J, Nietosvaara Y, Hurmerinta K, Suominen E (2002) Microvascular temporomandibular joint and mandibular ramus reconstruction in hemifacial microsomia. J Craniofac Surg 13: 809–815
231. Vinkka H (1982) Secondary cartilages in the facial skeleton of the rat. Proc Finn Dent Soc 78(suppl 7): 1–137
232. Wang L, Detamore MS (2007) Tissue engineering the mandibular condyle. Tissue Eng 13:1955–1971
233. Weinberg SM, Neiswanger K, Martin RA, Mooney MP, Kane AA, Wenger SL, Losee J, Deleyiannis F, Ma L, De Salamanca JE, Czeizel AE, Marazita ML (2006) The Pittsburgh Oral-Facial Cleft study: expanding the cleft phenotype. Background and justification. Cleft Pal Craniofac J 43:7–20
234. Wilkin DJ, Mortier GR, Johnson CL, Jones MC, de Paepe A, Shohat M, Wildin RS, Falk RE, Cohn DH (1998) Correlation of linkage data with phenotype in eight families with Stickler syndrome. Am J Med Genet 80:121–127
235. Winnier G, Blessing M, Labosky PA, Hogan BL (1995) Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. Genes Dev 9:2105–2116
236. Wong FK, Hagg U (2004) An update on the aetiology of orofacial clefts. Hong Kong Med J 10:331–336
237. Wright DM, Moffett B (1974) The postnatal development of the human temporomandibular joint. Am J Anat 141:235–249
238. Wyszynski DF, Duffy DL, Beaty TH (1997) Maternal cigarette smoking and oral clefts: a meta-analysis. Cleft Pal Craniofac J 34:206–210
239. Yanagisawa H, Clouthier DE, Richardson JA, Charite J, Olson EN (2003) Targeted deletion of a branchial arch-specific enhancer reveals a role of dHAND in craniofacial development. Development 130:1069–1078
240. Yu L, Gu S, Alappat S, Song Y, Yan M, Zhang X, Zhang G, Jiang Y, Zhang Z, Zhang Y, Chen Y (2005) Shox2-deficient mice exhibit a rare type of incomplete clefting of the secondary palate. Development 132:4397–4406
241. Yu W, Ruest L, Svoboda K (2009) Regulation of epithelial-mesenchymal transition in palatal fusion. Exp Biol Med 234(5):483–491
242. Yuodelis RA (1966) Ossification of the human temporomandibular joint. J Dent Res 45:192198
243. Zhang FP, Mikkonen L, Toppari J, Palvimo JJ, Thesleff I, Janne OA (2008) Sumo-1 function is dispensable in normal mouse development. Mol Cell Biol 28:5381–5390
244. Zhu H, Curry S, Wen S, Wicker NJ, Shaw GM, Lammer EJ, Yang W, Jafarov T, Finnell RH (2005) Are the betaine-homocysteine methyltransferase (BHMT and BHMT2) genes risk factors for spina bifida and orofacial clefts? Am J Med Genet A 135:274–277
245. Frebourg T, Oliveira C, Hochain P, Karam R, Manouvrier S, Graziadio C, Vekemans M, Hartmann A, Baert-Desurmont S, Alexandre C, Lejeune Dumoulin S, Marroni C, Martin C, Castedo S, Lovett M, Winston J, Machado JC, Attie T, Jabs EW, Cai J, Pellerin P, Triboulet JP, Scotte M, Le Pessot F, Hedouin A, Carneiro F, Blayau M, Seruca R (2006) Cleft lip/palate and cdh1/e-cadherin mutations in families with hereditary diffuse gastric cancer. J Med Genet 43(2):138-142.