The genetic basis of normal and abnormal craniofacial development

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In recent years our knowledge of the genetic mechanisms behind animal development has increased exponentially, and it has become apparent that these mechanisms have been conserved to an astonishing extent during evolution. In this review some important groups of developmental regulatory genes are introduced, and their roles are discussed in the context of craniofacial morphogenesis. Transcription factors regulating both the identity and patterning of embryonic structures and the development of individual organs are often called master regulatory genes. These genes, as well as other transcription factors, are parts of signaling networks mediating cellular communication, including inductive interactions between nearby tissues. Experimental studies, in particular the genetic analysis of mouse development, continue to demonstrate important roles for increasing numbers of these developmental regulatory molecules, including the actual signals, their receptors, and transcription factors in the development of the jaws, cranial bones, and teeth. Molecular genetic studies have shown that mutations in the genes of the signaling networks cause a variety of human craniofacial defects. □ *Bone development; craniofacial defects; hypodontia; signaling networks; tooth development*

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Embryonic development is controlled by genes inherited from our parents. It is believed that a significant proportion of the 80,000–100,000 genes in human beings have functions in the regulation of embryogenesis. The secrets of development will not be resolved only by identifying and sequencing the genes that are involved. We need to understand the functions of individual genes and, in particular, the mechanisms whereby the expression of genes is regulated in time and space.

The rapid advancement of gene technology, which has caused a revolution in all fields of biomedical research during the last 10 years, has affected research in developmental biology in a particularly dramatic way. It is now possible to examine development at the level of genes, and increasingly sophisticated methods allow genetic manipulations of cells and tissues in vitro and of whole animals in vivo. The production of transgenic mice has proven to be an extremely powerful method in unraveling the developmental functions of individual genes. As a consequence we are starting to understand details of the molecular mechanisms regulating various aspects of embryonic development, including the three-dimensional patterning of the whole embryo and the positions of different structures, the morphogenesis of individual organs, and the differentiation of various cell types. Craniofacial development presents particular challenges because of its great complexity. The head and face comprise a multitude of different structures, and their development must be intricately synchronized. Good examples of such coordination are the development of muscles and their attachment to bones and the development of teeth and alveolar bone.

Developmental regulatory genes

Many different types of genes are involved in developmental regulation, and specific categories are currently of particular interest: specific transcription factors, which are often called master regulatory genes and genes of signaling networks.

Master regulatory genes

The master regulatory genes encode transcription factors, that is, proteins that regulate the expression, or transcription, of genes in the nucleus. Transcription factors are grouped according to the regions in the molecules that mediate their binding to DNA. Homeobox-containing transcription factors have been under extremely active study since they were first found in the fruit fly *Drosophila melanogaster* and then in mice and humans in the mid-1980s (1). Anteroposterior patterning of embryos in all animals—including worms, flies, fish, and man—is regulated by a specific set of homeobox-containing genes. Between different animals these Hox cluster genes show remarkable similarity in their sequence, demonstrating that they have been conserved to a previously unexpected degree during evolution. This conservation has since been shown to be a characteristic of all master regulatory genes. The Hox cluster genes are not expressed anteriorly to the second branchial arch, and hence they do not regulate the development of the face and teeth, but numerous other homeobox-containing genes are important regulators of craniofacial development. For example, *Msx* and *Dlx* genes as well as the genes *goosecoid* and

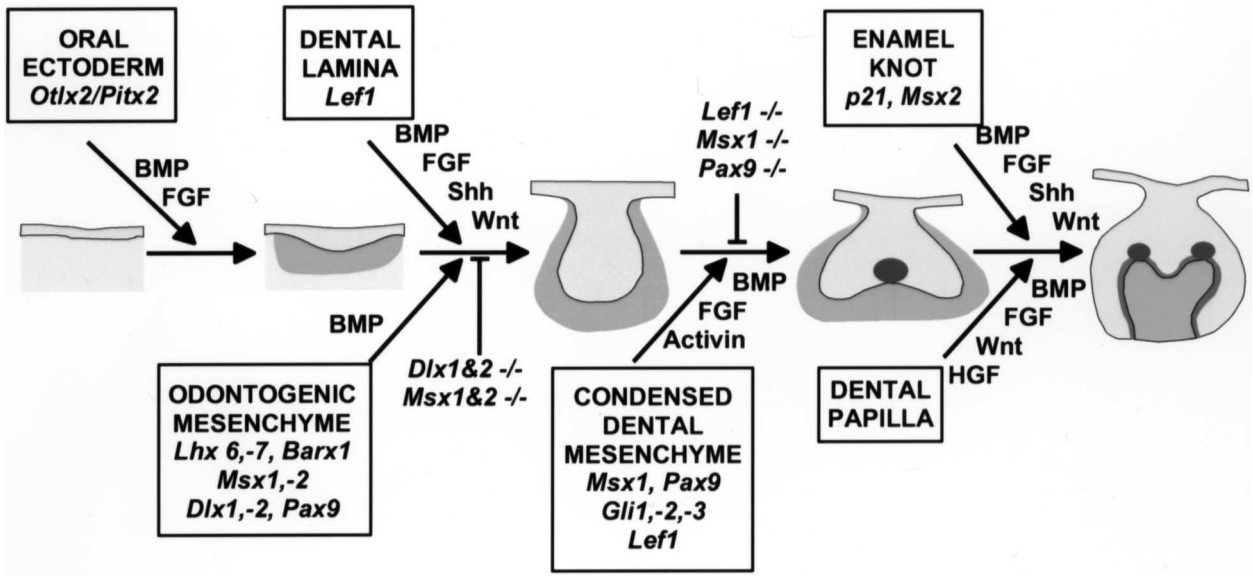

Fig. 1. Sequential and reciprocal signaling between the epithelium and mesenchyme regulates tooth morphogenesis. The signals belong to conserved families regulating cellular communication in practically all tissues and organs. The epithelial dental lamina signals to the mesenchyme during tooth initiation, and thereafter the mesenchyme regulates epithelial morphogenesis. Shape development is regulated by signals from the epithelial enamel knot and the dental papilla mesenchyme. Transcription factors expressed in the signaling tissues are depicted in the boxes. Tooth development is arrested at the bud stage in mice with deficient function of the transcription factors *Msx1, Lef1*, and *Pax9*. The arrest occurs before bud stage when both *Dlx1* and *Dlx2*, or *Msx1* and *Msx2*, are inactivated. BMP = bone morphogenetic protein; FGF = fibroblast growth factor; Shh = sonic hedgehog; HGF = hepatocyte growth factor. (Courtesy of Thomas Åberg.)

MHox regulate the development of the first branchial arch (2–5).

Many transcription factors have master regulatory functions in the initiation and morphogenesis of individual organs. A good example is *Pax6*, a transcription factor containing a paired box, which initiates eye development in all different animals analyzed (6). *Lim1*, a lim-domain–containing transcription factor, is required for head formation, as demonstrated in mice that lack this gene and, quite dramatically, develop without heads (7). Tooth development also depends on several key transcription factors. These include the homeobox genes *Dlx1, Dlx2, Msx1,* and *Msx2*, the paired box gene *Pax9*, and *Lef1*, a HMG-group transcription factor (8–11). If the function of these is abolished in mouse embryos, tooth development is arrested before or at the bud stage (Fig. 1). Tooth morphogenesis also requires the function of the runt-domain–containing transcription factor *cbfa1/osf2* (12), which, interestingly, is a master control gene of osteoblast function and bone development and the causative gene in cleidocranial dysplasia (13, 14; see below).

Genes in signaling networks

Communication between nearby cells constitutes a central mechanism by which the advancing development of the embryo is regulated. This communication (also called embryonic induction) is mediated by complex signaling networks (Fig. 2). Numerous molecules participate in these networks; the inducing cell produces the actual signal substance, which binds to a specific receptor in the responding cell and thereby elicits a cascade involving many molecules inside the cell. The cascade ends in the nucleus, where activated transcription factors, including the master regulatory genes discussed above, regulate gene expression and thereby determine the nature of the cellular response to the signal. Besides transcription factors, other components of the signaling networks, such as the signal molecules and their receptors, have been highly conserved during evolution.

The signal molecules (many of them are called growth factors) belong to several families, and four of them have been particularly intensely studied during recent years: the hedgehogs (hh), the bone morphogenetic proteins (BMP) (15), the fibroblast growth factors (FGF), and the Wnt-family signaling molecules. One or more members of all of these four families have been detected in practically all organs that have been analyzed in detail. The tooth is a perfect example of such an organ (16). As shown in Fig. 1 these signals mediate communication between the epithelial and mesenchymal components of the developing tooth germ during several stages of development. Fig. 1 also shows the transcription factors, expressed at specific stages of development, that participate in the signaling networks. The expression patterns of these and other genes during tooth development can be examined in a graphic Internet database (17).

There are many examples of the powerful effects of these signal molecules. For instance, implantation of a bead releasing FGF to the flank region of a chick embryo

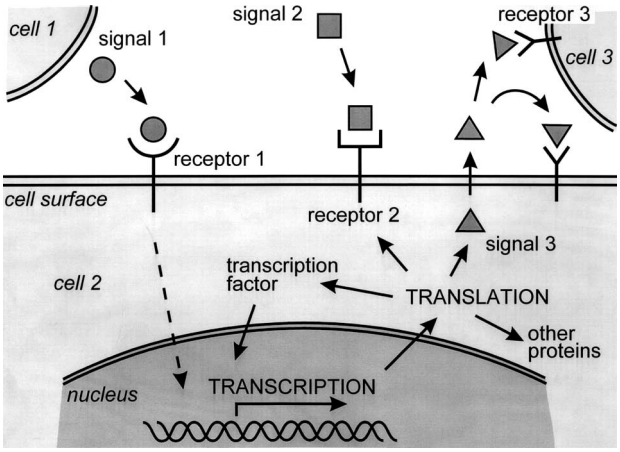

Fig. 2. Schematic presentation of signaling networks regulating cellular communication during development. Cell 1 expresses signal 1, which binds to its specific receptor on the surface of cell 2. This leads to an intracellular molecular cascade, which results in regulation of the transcription of target genes in the nucleus. In the cytoplasm new proteins are synthesized, and these may include a new receptor 2, which makes the cell responsive to a new signal 2. Also, the synthesis of a new signal 3 may be induced, mediating the communication of the cell with its neighbor cells expressing its receptor 3. In addition to new signals and receptors, transcription factors and many other molecules affecting the functions of the cell may also be activated as a result of the signal 1 binding to its receptor 1. (Courtesy of Carin Sahlberg.)

induces the development of extra limbs between the wing and the leg (18), and ectopic expression of Shh (sonic hedgehog) in the anterior part of the early limb bud induces duplication of the digits (19). Deficient function of several genes encoding FGFs, BMPs, and Wnts results in early embryonic lethality because they regulate early cell and tissue organization.

The development of bone and cartilage is also regulated by the same conserved signaling networks. As indicated by their name the BMPs were first found in bone, and they were shown to be potent inducers of *de novo* bone formation (20). Ihh (Indian hedgehog) regulates the development of epiphyseal cartilage in long bones, and it is involved in keeping the cartilage cells in an undifferentiated state and preventing their precocious differentiation (21).

An interesting recent example of a signal needed for the development of the mandible is ET-1 (endothelin). Mice in which genes in the ET-1 signaling pathway are knocked out develop with severely defective lower jaws. The ET-1 signal is expressed by the epithelium covering the mandibular arch, and the receptors are expressed by the neural crest–derived mesenchyme (22). ET-1 signaling was shown to be essential for the development of Meckel's cartilage.

Gene defects causing craniofacial malformations

Transgenic mouse technology, in particular the production of mice with deficient gene function, has pointed out the requirement of numerous genes for the development of individual embryonic structures and organs, as illustrated by the above examples. These observations have frequently led to the identification of defects in the same genes in human syndromes. In addition, gene defects causing congenital malformations in humans are being identified with increasing speed by using the advancing molecular genetic methods. It has turned out that defects in the master regulatory genes and in genes of the signaling networks are common causes of congenital defects, including craniofacial malformations (Table 1).

Although defects in the craniofacial hard tissues (bone, cartilage, dentin, enamel) in many cases result from disturbances in the extracellular matrix molecules, such as collagens and enamel proteins (32, 33), most craniofacial defects appear to be caused by defects in the developmental regulatory genes, including transcription factors and other molecules mediating intercellular communication. Examples of such syndromes include holoprosencephaly, which in some cases is caused by mutations in the Shh gene (23); craniosynostosis syndromes, which are caused by mutations in several different genes of the FGF signaling pathway (Table 1; see also Cohen & Kreiborg, this issue); and cleidocranial dysplasia, which

Table 1. Some human craniofacial defects caused by mutations in genes of signaling networks

Syndrome	Phenotype	Causative gene	Type of molecule	Ref.
Holoprosencephaly	Midface deficiency	*SHH*	Signal molecule	23
Apert	Craniosynostosis	*FGFR2*	Signal receptor	24
Pfeiffer	Craniosynostosis	*FGFR1*	Signal receptor	25
Crouzon	Craniosynostosis	*FGFR3*	Signal receptor	26
Boston type	Craniosynostosis	*MSX2*	Transcription factor	27
Saethre–Chotzen	Craniosynostosis	*TWIST*	Transcription factor	28
Cleidocranial dysplasia	Deficient bone, supernumerary teeth	*CBFA/OSF2*	Transcription factor	14
Oligodontia	Hypodontia	*MSX1*	Transcription factor	29
Rieger	Hypodontia, eye and umbilical defects	*RIEG/PITX2*	Transcription factor	30
Anhidrotic ectodermal dysplasia	Hypodontia, gland and hair hypoplasia	*EDA/ectodysplasin A*	Cell surface molecule	31

results from a loss of function mutation in the transcription factor *cbfa1/osf2*, a master control gene of bone formation (14, 34).

Some gene defects that cause hypodontia have recently been identified. Mutations in the *MSX1* gene cause oligodontia (29). Defects in another transcription factor, *RIEG/PITX2,* cause Rieger syndrome, which affects not only teeth but also eye and umbilical development (30). Anhidrotic ectodermal dysplasia (EDA) is caused by loss of function of ectodysplasin A, a cell surface molecule whose function is unknown but presumably involved in mediation of cell communication between the epithelium and mesenchyme (31).

Identification of the gene defects causing craniofacial malformations is the first step toward possible new forms of therapy and future prevention. The functions of these genes are now actively studied using experimental in vitro cell and tissue cultures and in vivo mouse models. For example, the spontaneous mouse mutation *Tabby* was shown to be the homologue of human EDA, and thus it can be used to study the function of ectodysplasin A (35). Another example is the *cbfa1/osf2* deficient transgenic mouse, which can be used to study various aspects of bone formation. The heterozygotes of this mutant and the radiation-induced *ccd* mouse mutation serve as models for human cleidocranial dysplasia (34, 36). The *cbfa1/osf2* knockouts have also been used to analyze the function of the gene in tooth morphogenesis (12). Hence, we can expect that the pathogenesis of many malformations will be clarified and that the roles of the mutated genes in the regulatory networks will be pinpointed.

This information may form the basis of both new treatment and prevention methods, probably by affecting specific steps in signaling networks. The use of BMPs in the stimulation of bone development is already being tested in clinical trials. There are also promising results from animal experiments in which the application of signal molecules has affected specific stages of morphogenesis and in some cases partially rescued development. Tooth development proceeded until cap stage in the *Msx1* knockout mouse embryos when their tooth buds were cultured in the presence of BMP protein. This rescue was based on the observation that one function of the *Msx1* gene in the tooth buds is to stimulate the production of BMP-4 (37). The closure of sutures in cultured mouse calvaria was stimulated by FGF protein (38), and sweat gland hypoplasia was partially rescued by EGF (epidermal growth factor) in *Tabby* mice (39).

References

1. Gilbert SF. Developmental biology. 5th ed. Sunderland (MA): Sinauer Associates; 1997.
2. Satokata I, Maas R. Msxl deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. Nat Genet 1994;6:348–56.
3. Yamada G, Mansouri A, Torres M, Stuart ET, Blum M, Schultz M, et al. Targeted mutation of the murine *goosecoid* gene results in craniofacial defects and neonatal death. Development 1995;121: 2917–22.
4. Qiu M, Bulfone A, Martinez S, Meneses JJ, Shimamura K, Pedersen RA, et al. 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 1995;9:2523–38.
5. Martin J, Bradley A, Olson E. The *paired*-like homeo box gene *Mhox* is required for early events of skeletogenesis in multiple lineages. Genes Dev 1995;9:1237–49.
6. Callaerts P, Halder G, Gehring WJ. PAX-6 in development and evolution. Ann Rev Neurosci 1997;20:483–532.
7. Shawlot W, Behringer RR. Requirement for *Lim1* in head-organizer function. Nature 1995;374:425–30.
8. Van Genderen C, Okamura RM, Farinas I, Quo RG, Parslow TG, Bruhn L, et al. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1 deficient mice. Genes Dev 1994;8:2691–703.
9. Qiu M, Bulfone A, Ghattas I, Meneses J, Sharpe PT, Presley R, et al. 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 first and second arches. Dev Biol 1997;185:165–84.
10. Peters H, Neubuser A, Balling R. Pax genes and organogenesis: *Pax9* meets tooth development. Eur J Oral Sci 1998;106:38–43.
11. Bei M, Maas R. FGFs and BMP4 induce both *Msx1*-independent and *Msx1*-dependent signaling pathways in early tooth development. Development 1998;125:4325–33.
12. D'Souza RN, Åberg T, Gaikwad J, Cavender A, Owen M, Karsenty G, et al. *Osf2* is required for epithelial-mesenchymal interactions regulating tooth morphogenesis in mice. In press 1999.
13. Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 1997;89:747–54.
14. Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, et al. Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. Cell 1997;89:773–9.
15. Hogan BL. Bone morphogenetic proteins in development. Curr Opin Genet Dev 1996;6:432–8.
16. Thesleff I, Nieminen P. Tooth morphogenesis and cell differentiation. Curr Opin Cell Biol 1996;8:844–50.
17. Developmental Biology Programme, University of Helsinki. Gene expression in tooth [Internet database]. 1998. Available from URL: http://honeybee.helsinki.fi/toothexp
18. Cohn MJ, Izpisua-Belmonte JC, Abud H, Heath JK, Tickle C. Fibroblast growth factors induce additional limb development from the flank of chick embryos. Cell 1995;80:739–46.
19. Tickle C. Vertebrate limb development. Curr Opin Genet Dev 1995;5:478–84.
20. Wozney J. The bone morphogenetic protein family and osteogenesis. Mol Reprod Dev 1992;32:160–7.
21. Kronenberg HM, Lee K, Lanske B, Segre GV. Parathyroid hormone-related protein and Indian hedgehog control the pace of cartilage differentiation. J Endocrinol 1997;154 Suppl:S39–45.
22. Clouthier DE, Hosoda K, Richardson JA, Williams SC, Yanagisawa H, Kuwaki T, et al. Cranial and cardiac neural crest defects in endothelin-A receptor-deficient mice. Development 1998;125:813–24.
23. Roessler E, Belloni E, Gandenz K, Jay P, Berta P, Scherer SW, et al. Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. Nat Genet 1996;14:357–60.
24. Wilkie AOM, Slaney SF, Oldridge M, Poole MD, Ashworth GJ, Hockley AD, et al. Apert syndrome results from localized mutations of FGFR2 and is allelic with Crouzon syndrome. Nat Genet 1995;9:165–72.
25. Muenke M, Schell U, Hehr A, Robin NH, Losken HW, Schinzel A, et al. A common mutation in the fibroblast growth factor receptor 1 in gene Pfeiffer syndrome. Nat Genet 1994;8:269–74.

26. Jabs EW, Li X, Scott AF, Meyers G, Chen W, Eckles M, et al. Jackson-Weiss and Crouzon syndromes are allelic with mutations in fibroblast growth factor receptor 2. Nat Genet 1994;8:275–9.
27. Howard TD, Paznekas WA, Green ED, Chiang LC, Ma N, Ortiz de Luna RI, et al. Mutations in TWIST, a basic helix-loop-helix transcription factor, in Saethre-Chotzen syndrome. Nat Genet 1997;15:36–41.
28. el Ghouzzi V, Le Merrer M, Perrin-Schmitt F, Lajeunie E, Benit P, Renier D, et al. Mutations of the TWIST gene in the Saethre-Chotzen syndrome. Nat Genet 1997;15:42–6.
29. Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE. A human MSX1 homeodomain missense mutation causes selective tooth agenesis. Nat Genet 1996;13:417–21.
30. Semina EV, Reiter R, Leysens NJ, Alward WL, Small KW, Datson NA, et al. Cloning and characterization of a novel bicoid-related homeobox transcription factor gene, RIEG, involved in Rieger syndrome. Nat Genet 1996;14:392–9.
31. Kere J, Srivastava AK, Montonen O, Zonana J, Thomas N, Ferguson B, et al. X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. Nat Genet 1996;13:379–80.
32. Spranger J, Winterpacht A, Zabel B. The type II collagenopathies: a spectrum of chondrodysplasias. Eur J Pediatr 1994; 153:56–65.
33. Forsman S, Lind L, Bäckman B, Westermark E, Holmgren G. Localization of a gene for autosomal dominant amelogenesis imperfecta (ADAI) to chromosome 4q. Hum Mol Genet 1994; 3:1621–5.
34. Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, et al. Cbfa 1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell 1997;89:765–71.
35. Srivastava A, Pispa J, Hartung P, Du Y, Ezer S, Jenks T, et al. Tabby phenotype is caused by mutation in mouse homologue of EDA gene, which reveals novel human exons and encodes a protein (ectodysplasin-A) with collagenous domains. Proc Natl Acad Sci U S A 1997;94:13069–74.
36. Huang L-F, Fukai N, Selby PB, Olsen BR, Mundlos S. Mouse clavicular development: analysis of wild-type and cleidocranial dysplasia mutant mice. Dev Dyn 1997;210:33–40.
37. Chen Y, Bei M, Woo I, Satokata I, Maas R. Msx1 controls inductive signaling in mammalian tooth morphogenesis. Development 1996;122:3035–44.
38. Kim H-J, Rice DPC, Kettunen PJ, Thesleff I. FGF, BMP, and Shh mediated signalling pathways in the regulation of cranial suture morphogenesis and calvarial bone development. Development 1998;125:1241–51.
39. Blecher SR, Kapalanga J, Lalonde D. Induction of sweat glands by epidermal growth factor in murine X-linked anhidrotic ectodermal dysplasia. Nature 1990;345:542–4.