Homeobox genes and growth factors in regulation of craniofacial and tooth morphogenesis

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Homeobox genes encode a special group of transcription factors that regulate gene expression in the developing embryo. The so-called Hox-cluster genes were first discovered in the *Drosophila* (fruit fly). They specify the identity of body segments and their patterning along the anteroposterior axis. Other homeobox-containing genes appear to regulate patterning of the head and face. The function of the *Msx-1* homeobox gene has been shown to be necessary for tooth development. In general, it is thought that special combinations of homeobox genes specify the patterning of individual structures. Bone morphogenetic proteins (BMPs) are growth factors belonging to the family of transforming growth factor-beta (TGF-β). BMPs regulate bone and cartilage development, and individual BMPs have been shown to contribute to the shaping of various skeletal elements. BMPs regulate bone and dentin formation also postnatally, and they have therapeutic potential in reparative osteogenesis and odontogenesis. BMPs also act as inductive signals between tissue layers in the embryo, and they regulate the expression of several transcription factors, including homeobox-containing genes. BMP-4 has been identified as an epithelial inductive signal in tooth development. As it is produced by early dental epithelium and regulates tooth-specific gene expression in the dental mesenchyme, including Msx-1 expression, it may be an important signal for the initiation of tooth development. □ *Bone morphogenetic proteins; embryology; odontogenesis; transgenic mice*

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Morphogenesis involves the sequence of events that determine the form and structure of an organism. At the level of single cells, advancing development is reflected predominantly in their proliferation and differentiation. Cell migration also plays an important role during some developmental events, and in others the cells undergo programed cell death, or apoptosis, which contributes to localized removal of tissue and shaping of organs (1). The central question in developmental biology is, what are the mechanisms that regulate the behavior or, in other words, gene expression, of cells so that they participate in their characteristic manner in the morphogenetic program. Of specific interest here are the mechanisms that define the position and the state of differentiation of individual cells, and the mechanisms whereby cells are induced to become different from other cells.

During the past 10 years our knowledge about the molecules controlling prenatal development in general, and craniofacial development in particular, have increased tremendously. This has been due to the revolution in molecular biology. As it is not possible to cover all molecules involved in craniofacial development in a short paper, I have decided to focus on two special groups of molecules, namely the homeobox-containing transcription factors and growth factors. Within these groups I shall discuss a few individual molecules, which are now known to play important regulatory roles in craniofacial and/or tooth development.

Transcription factors

Transcription factors are nuclear proteins that regulate the expression of genes by binding to DNA. A vast number of transcription factors have been described, and it is obvious that most of them regulate gene expression also in the developing embryo. Transcription factors bind to DNA by different mechanisms, and one special group of them, which binds to DNA via the so-called homeodomain, appears to have a key role in developmental regulation.

Homeobox-containing genes

The genes of this special group of transcription factors all contain the homeobox, a 180-base-pair sequence that encodes the DNA-binding homeodomain. The special feature of these genes is that at least some of them appear to encode positional information during embryogenesis. Hox genes were first detected in the fruit fly (*Drosophila*), in which they were defined as major pattern formation genes. A remarkable example of the potency of Hox genes in regulating position is a fly in which the mutation in the homeobox-containing *Antennapedia* gene causes the development of legs in place of the antennae. It was also discovered that the homeobox genes were organized as two clusters in one *Drosophila* chromosome so that their position correlated to the anteroposterior sequence of expression (1, 2).

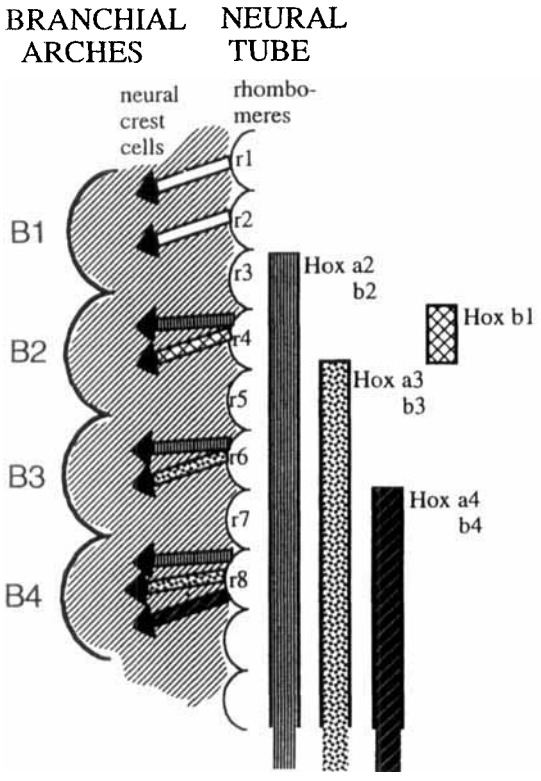

Fig. 1. Schematic presentation of migration of neural crest cells to the branchial arches and expression of homeobox genes. The migrating cells continue to express the same homeobox genes as their precursors express in the rhombomeres, and so the 'hox code' of the hindbrain is transferred to the branchial arches. Apparently, the neural crest cells are partly specified before migration. Modified from Ref. 16.

A major breakthrough was the discovery that the mouse and also other vertebrates including humans have homeobox genes and that they specify positional information. The vertebrate Hox genes have arisen during evolution by duplication of the ancestral genes, and they are organized in four separate clusters (a–d) on different chromosomes (3). In vertebrates there is also correspondence between the sequence of the genes and their relative expression along the a–p axis. The individual Hox-cluster genes are named in accordance with the cluster (a–d) and the relative position within the cluster (3) (Fig. 1).

The anterior limits of expression of individual Hox genes are important for their function, and it is believed that combinations of different Hox genes, expressed in specific locations, form 'Hox codes' which specify the position of cells. The generation of transgenic mice in which either a Hox gene is overexpressed (that is, it is expressed also in cells that would not express it normally) or in which a Hox gene has been made non-functional (so-called knockout mutants) has resulted in homeotic transformations (4). In these mice, the identity of body segments is altered so that anterior structures are transformed to posterior ones, or vice versa. Dramatic examples are mice in which the disruption of the *Hox a-2* gene caused the lack of derivatives of the second branchial arch and a change in the identity of some second-arch structures to first-arch structures (5). For instance, the stapes was missing, but a supernumerary malleus had developed, and, in addition a truncated ectopic Meckel's cartilage was evident. Most of the embryos had cleft palate, which, however, was thought to have arisen as a secondary consequence of changes in the more caudal skeletal elements (5). The *Hox a-2* knockout mouse clearly demonstrates the power of Hox genes to regulate pattern formation, and the number of similar transgenic mouse experiments is increasing. For instance, ectopic expression of the *Hox d-4* gene results in transformation of occipital bones into vertebrae (6), and in the double mutants, in which *Hox a-3* and *Hox d-3* are disrupted, the atlas is deleted (7). It is now generally believed that changes in Hox gene expression have been a central feature in evolution.

Neural crest cells that migrate from the neural tube into the craniofacial region and branchial arches give rise to most skeletal elements in these areas (8–13). Studies analyzing the migration patterns and Hox gene expression of the neural crest cells suggest that the neural crest cells continue to express their specific Hox genes during migration (Fig. 1) (8, 14). The phenotype of the Hox a-2 knockout mouse, described above, suggests that the neural crest cells are at least partly specified before migration. This was earlier shown to be the case by Noden (15), who used transplantation experiments between quail and chick tissues. When those neural crest cells that give rise to the first branchial arch were transplanted before their migration into the area of second arch neural crest, duplicated first-arch structures appeared, including an ectopic beak. Hence, the phenotype of the chimeric chick resembled that of the *Hox a-2* knockout mouse.

An important conclusion from the present available data is that in the branchial arches, each arch expresses a different and arch-specific combination of Hox genes, and this combinatorial code is involved in transmitting positional or morphogenetic specification from the hindbrain to the branchial arches (8, 14, 16). No expression of the Hox-cluster genes has, however, so far been detected in the brain or neural crest anterior to the third rhombomere in the hindbrain or second branchial arch, respectively (Fig. 1). This is thought to be associated with the evolution of the vertebrate head (9, 12).

Besides the Hox-cluster genes there are numerous other transcription factors that contain the homeodomain. These genes are believed to regulate patterning in the craniofacial area and in different organs, and some of them are thought to act as regulators of cell fate. For instance, the distributions of *Tlx* and *Dlx* genes suggest roles in the patterning of structures in the brain and craniofacial region (17–19). The patterns of expression of many homeobox genes have suggested

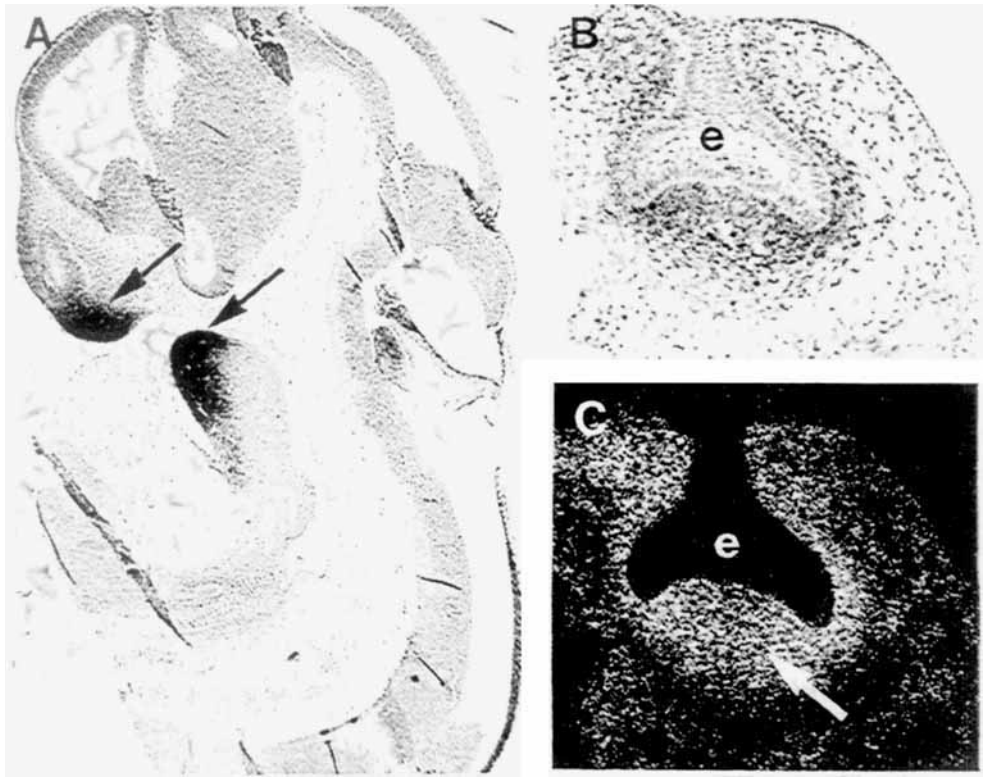

Fig. 2. Expression of the homeobox gene *Msx-1* in a mouse embryonic facial processes. In the in situ hybridization analysis, the bound ^{35}S-labeled cDNA probe is detected by autoradiography. 2A. Sagittal section of a 12-day-old embryo. Intense expression is seen in the neural crest-derived mesenchymal cells of the frontonasal and mandibular processes (arrows). 2B and C. In a cap-stage tooth germ, *Msx-1* is expressed intensely in the mesenchymal cells of the dental papilla and dental sac. Expression of the *Msx-1* gene is necessary for tooth development as transgenic mice lacking a functional *Msx-1* gene have no teeth (22) (e = dental epithelium). 2C shows the section in Figure B in dark-field illumination.

functions in development of many organs, and by now there are several examples in which such roles have been confirmed either by experimentally manipulating the mouse homeobox genes (transgenic mice) or by analysis of mutations in human syndromes. The homeobox-containing *Pax-3* gene was shown to be mutated in the Waardenburg syndrome, which is a defect of neural crest cells (20). The Boston-type craniosynostosis was shown to be caused by a mutation in the homeodomain of the *Msx-2* gene (21).

The homeobox-containing gene *Msx-1* was recently shown to be necessary for normal development of the secondary palate and tooth (22). Transgenic mice in which the *Msx-1* gene had been made non-functional had cleft palate and complete anodontia. Tooth development had been arrested at the bud stage. *Msx-1* is intensely expressed in the neural crest-derived mesenchymal cells in the facial processes (Fig. 2A) and is especially intense in the dental mesenchyme throughout tooth morphogenesis (23) (Fig. 2B, C). On the other hand, *Msx-1* is intensely expressed also in the limbs, but no limb defects were seen in the mice lacking a functional *Msx-1* gene (22). This indicates, as do the phenotypes of many other knockout transgenic mice, that the patterns of gene expression are not necessarily indicative of the developmental functions of genes.

Growth factors

Growth factors constitute an important class of signaling molecules. Unlike hormones that act over long distances through an endocrine mechanism, growth factors exert their effects locally. A growth factor produced by one cell may either affect the behavior of another cell in its vicinity, in which case the effect is defined as paracrine, or it may affect the same cell that is producing it in an autocrine way. The effects of growth factors are always mediated through binding of the factor to specific cell surface receptors. The signaling cascade from the cell surface to the nucleus of the cell is extremely complex and constitutes a very active field of research.

Growth factors are grouped into families on the basis of similarities in structure (Table 1), and new factors are being discovered constantly. Growth factors in different families bind to their characteristic cell surface recep-

Table 1. Growth factor families*

Transforming growth factor-beta (TGF-β)
TGFβ 1–5, bone morphogenetic protein (BMP) 2–8, growth and differentiation factor (GDF) 1–7
Epidermal growth factor (EGF)
EGF, TGFα, amphiregulin, HB-EGF
Fibroblast growth factor (FGF)
FGF 1–8
Insulin-like growth factor (IGF)
IGF 1–2
Platelet-derived growth factor (PDGF)
PDGF A, B
Neurotrophins
Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT) 3–4

* Some growth factors in all these families have been implicated in craniofacial and tooth morphogenesis.

tors, which show also great variability. In some cases, many growth factors in the family use the same receptor, whereas in other families individual factors may bind to several different receptors. Sometimes additional binding to non-signaling co-receptors is also required for signal transduction. This obviously creates much variability in the regulation of responsiveness of cells to growth factors.

During embryonic development many growth factors have been shown to act as signals between tissue layers. Tissue interactions, also called embryonic induction, are a central mechanism of developmental regulation (24). Signaling functions for growth factors were first demonstrated in the primary embryonic inductive events involving formation of mesoderm and neural tissue (25). Now it is clear that the same growth factors, notably those in transforming growth factor-beta (TGF-β) and fibroblast growth factor (FGF) families act as signals also during organogenesis (Fig. 3) (26, 27). One mechanism whereby growth factors regulate development is through stimulation of homeobox genes (28, 29). Like the Hox genes, growth factors have also been conserved during evolution and appear to have similar functions across the animal kingdom. The family that has been most intensively studied in this respect is the TGF-β family, and within this family the so-called bone morphogenetic proteins are now believed to have very potent developmental regulatory functions.

Bone morphogenetic proteins (BMPs)

The name BMP was given to the active substance in bone and dentin powder that stimulated the formation of new bone when implanted in muscle (30). The activity was purified, and subsequently several related proteins have been cloned (31, 32). At present at least

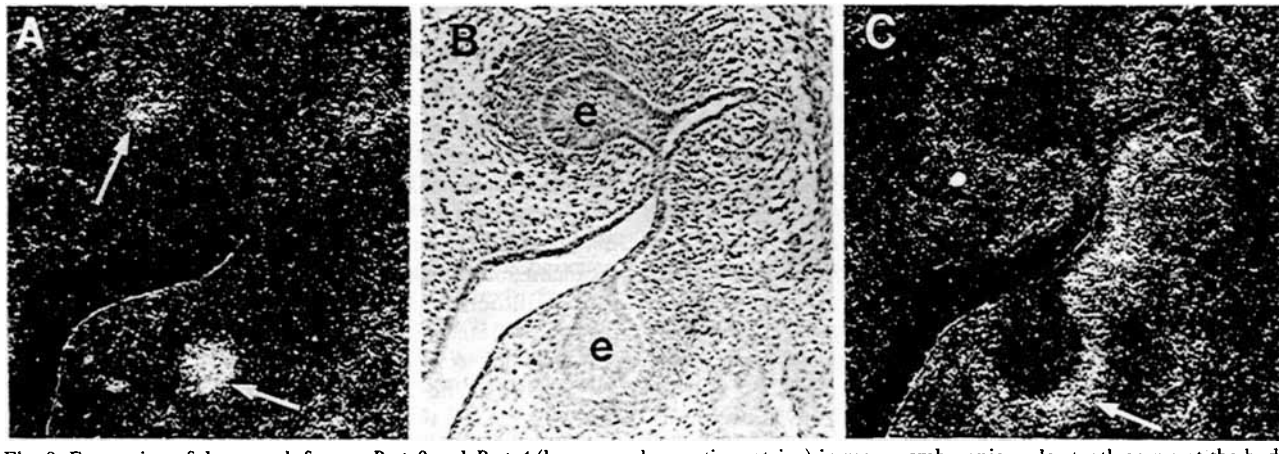

Fig. 3. Expression of the growth factors *Bmp-2* and *Bmp-4* (bone morphogenetic proteins) in mouse embryonic molar tooth germs at the bud stage. 3A. *Bmp-2* is expressed in the dental epithelium (arrows). 3B. A comparable section to those in A and C, as seen in bright-field illumination. 3C. *Bmp-4* is expressed in mesenchymal cells (arrow). BMPs have been identified as signals mediating interactions between embryonic cells, and in the early tooth germ they appear to be epithelial signals that instruct mesenchymal cell differentiation (37).

eight BMPs have been cloned, and all that have been tested exert bone and cartilage-inducing activity. It was quite unexpectedly discovered that the BMPs were homologous to growth factors that had earlier been shown to act as signals during *Drosophila* development. Now it is known that the *Drosophila dpp* gene is homologous to the vertebrate *Bmp-2* and *Bmp-4*. The great degree of conservation is evidenced by the potency of *Drosophila* dpp protein to induce endochondral bone formation in mammals (33).

BMPs are expressed in the condensed mesenchymal cells of bone primordia, and it appears that different BMPs are expressed in different bones. Recently, several new growth factors belonging to the related growth and differentiation factor (GDF) family have been cloned, and these are also expressed in developing bones (34). The morphogenetic roles of individual BMPs and GDFs in bone development have recently been documented. A mutation in the *Bmp-5* gene is the cause of the mouse short ear skeletal mutation (35). In addition to short ears, these mutant mice have characteristic abnormalities in the shapes of several bones, including vertebrae and ribs. The mouse mutation *brachypodism*, on the other hand, is caused by the *Gdf-5* gene (34). In these mice the long bones are shortened, and there are changes in the shape of the tarsals and metatarsals. These studies suggest that the specific shape of individual bones may be determined by the expression of particular BMPs and GDFs or, perhaps, by their combined effects.

BMPs are now known to act as inductive signals between tissue layers in the embryo (26). Dpp has such signaling functions in *Drosphila* embryos, and BMP-4 signals during formation of the mesoderm in vertebrate embryos (36). BMPs transmit signals also during so-called secondary induction, which constitutes interactions between epithelial and mesenchymal tissues in developing organs. The first evidence has come from studies on the developing tooth, in which BMPs, produced by the early dental epithelium, regulate the behavior of the underlying mesenchymal cells (Fig. 3) (37). In these experiments agarose beads were loaded with BMP proteins and placed on dental mesenchymal tissue in organ culture. The beads mimicked the effects of dental epithelium so that they induced the expression of the same genes as the dental epithelium, in particular the homeobox-containing *Msx-1* and *Msx-2* genes. As was mentioned above, lack of *Msx-1* expression is now known to lead to inhibition of tooth development (22). Hence, it can be speculated that the induction of *Msx-1* in dental mesenchyme by BMPs is a critical event for early tooth morphogenesis.

Concluding remarks

The significance of homeobox-containing transcription factors for craniofacial development has been demonstrated in several ways, and it can be expected that new genes will be cloned with increasing speed and that the Hox codes for various craniofacial structures will be discovered and deciphered in the future. The challenge is now also to study how homeobox genes are regulated and, on the other hand, what downstream genes are targets of individual homeobox-containing genes. There is evidence that retinoids may regulate the expression of Hox-cluster genes. For instance, ectopic expression of *Hox a-7* (earlier called *Hox1.1*) in transgenic mice has similar effects on phenotype as teratogenic exposure to retinoic acid (38). There is also evidence that growth factors regulate the expression of homeobox genes. The regulation of *Msx-1* expression in the developing tooth by BMP-2 and BMP-4 is one example of this (37). On the other hand, homeobox genes regulate growth factor genes, which demonstrates the complexity of the gene regulatory loops involved in development (16).

It is obvious that BMPs regulate craniofacial development in many ways. The different BMP genes are important for the determination of bone shape and for organ morphogenesis. In particular, BMPs appear to regulate the initiation and morphogenesis of teeth. BMPs have specific roles in the regulation of hard tissue development, and their therapeutic use as stimulators of new bone and dentin formation will offer new possibilities for clinical dentistry in the near future.

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