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Scientific Section

Construction for the Modern Head: current concepts in craniofacial development

MARTYN T. COBOURNE, B.D.S. (HONS), F.D.S.R.C.S. (ENG.), M.SC. (U.LOND), M.ORTH. R.C.S. (ENG.)

Department of Craniofacial Development/Department of Orthodontics, G. K. T. Dental Institute, King's College London, London Bridge, London SE1 9RT, UK

Abstract. *The vertebrate head is a highly complex composite structure whose morphological characteristics are controlled at the level of the gene. There is now increasing evidence for the role of gene families that encode transcription factors in determining the embryonic plan of the developing craniofacial complex. These genes act as regulators of gene transcription being intimately involved with the control of complex interactions between multiple downstream genes. Combinatorial expression of the* Hox *genes (a family of highly conserved master regulatory genes related to the homeotic genes of the fruitfly* Drosophila*) have been shown to play a definitive role in patterning distinct regions of the craniofacial complex. In the vertebrate,* Hox *genes pattern the hindbrain and branchial regions of the developing head up to and including structures derived from the second branchial arch. The first branchial arch and more rostral regions of the head are patterned by groups of homeobox genes more diverged from the original Hox clusters. Transgenic mice, with targeted disruptions in many of these genes, are now providing insights into the molecular mechanisms that lie behind a number of craniofacial defects seen in man.*

Index Words: Craniofacial development, *Hox* genes, Patterning.

Introduction

The study of embryology over the latter part of the last century has led to significant advances in our understanding of the many processes that are involved in generating an embryo. It is only recently, however, that the underlying mechanisms involved at a molecular level are beginning to be elucidated. The advent of molecular biology has allowed biologists to uncover, characterize, and ultimately manipulate the genes that make up the genome of the fertilized egg. We can now study how genes and proteins operate within their natural habitats. This is significantly furthering our understanding of the fundamental principles of development, how genes control cell behaviour and, thus, how they determine the pattern and form of an embryo. Without this knowledge of gene activity and the relevant cellular signal transduction pathways, elucidating the mechanisms that control development would be impossible. These advances are now influencing medicine and clinical genetics with almost daily progression in explaining the basis of a multitude of congential malformations, and abnormalities. It is important that clinicians attempt to keep abreast of these developments and orthodontists are not immune.

By virtue of the structure itself, generation of the craniofacial complex is a process that requires considerable organization. The vertebrate head is a composite structure whose formation begins early in development, as the brain is beginning to form. Central to the development of a head is the concept of segmentation, manifest in the hindbrain and branchial arch systems. In conjunction with migrating neural crest cells these systems will give rise to much of the head and neck and their associated, individualized compartments. It is now becoming clear that the molecular control of embryonic morphology resides at the level of the gene, in particular, within families of genes that encode transcription factors capable of regulating downstream gene transcription. This review aims to give an overview of some recent advances in our understanding of how these essential genes regulate the morphogenesis of such a complex structure as the head. It is not intended to be comprehensive and it concentrates on generally accepted principles, rather than the very latest science. This is, however, a rapidly changing field.

Correspondence: Email: martyn.cobourne@kcl.ac.uk

The Role of the Neural Crest

The neural crest is a highly pluripotent cell population that plays a critical role in the development of the vertebrate head. Unlike most parts of the body, the facial mesenchyme is derived principally from the neural crest and not the mesoderm of the embryonic third germ layer. In mammals, neural crest cells are formed during neurulation when cells at the margins of the neural folds undergo an epithelial to mesenchymal transition following an inductive interaction between neural plate and presumptive ectoderm. Neural crest cells migrate extensively throughout the embryo in four overlapping domains (cephalic, trunk, sacral, and cardiac), in the developing head the cephalic neural crest migrates from the posterior midbrain and hindbrain regions into the branchial arch system. The ectomesenchymal neural crest cells then interact with epithelial and meso-

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dermal cell populations present within the arches, leading to the formation of craniofacial bones, cartilages and connective tissues (Table 1). These pathways of neural crest migration, and ultimately the embryonic origin of the head and neck, have been extensively studied using a variety of cell labelling techniques (Noden, 1988; Couly and Le Douarin, 1987, 1990). More recently, a two-component genetic system has been used to indelibly mark the progeny of cranial neural crest during tooth and mandibular development (Chai *et al.*, 2000).

Advances have also been made in our understanding of how morphogenesis of the different regions of the craniofacial complex is mediated. There is now substantial evidence for the existence of highly conserved combinatorial gene codes, ultimately responsible for patterning different regions of the developing head and neck.

Patterning the Branchial Regions of the Head

Fundamental to the development of the craniofacial complex is the central nervous system (CNS). The CNS arises from the neural plate, a homogenous sheet of epithelial cells that forms the dorsal surface of the gastrula stage embryo. As the neural plate rolls up along its AP axis to form the neural tube the enlarged anterior end partitions into three vesicles. These vesicles are the primordia of the developing forebrain (prosencephalon), midbrain (mesencephalon), and hindbrain (rhombencephalon). It is the rhombencephalic derived neural crest that will give rise to the majority of the branchial arch mesenchyme. Migration of these populations of neural crest cells from the regions of the rhombencephalon results in a ventral relocation to within the branchial arches. Development of the mid- and lower regions of the craniofacial complex is intimately associated with these branchial regions. It is clear, therefore, that the neural crest derived from the hindbrain is essential for normal formation of the face and neck.

TABLE 1 *Derivatives of the cranial neural crest*

	Derivatives
Nervous system	
Neurons, including	Sensory ganglia
	Sympathetic ganglia (V, VII, IX, X)
	Parasympathetic ganglia of neck
	Neuroglial cells
	Schwann cells
Skeletal system	
Branchial arch cartilages	
Bones, including	Maxilla
	Mandible
	Palatine
	Facial complex
	Cranial vault
Connective tissues	
Connective tissue component of:	Cranial musculature
	Adenohypophysis
	Lingual glands
	Thymus
	Thyroid and parathyroids
Vascular and dermal smooth muscles	
Odontoblasts and pulp of the teeth	
Corneal endothelium and stroma	
Melanocytes and melanopores	
Pigment cells	
Epidermal pigment cells	
Secretory cells	
Carotid body Type I cells	
Calcitonin producing cells of ultimobranchial body	

Adapted from Ferguson (1993).

The hindbrain itself is known to be a segmented structure composed of eight subunits called rhombomeres (Lumsden and Keynes, 1989). Rhombomeres are important segmental units of organization, which have distinct morphological properties that vary with a two-segment periodicity. Each rhombomere represents a lineage-restricted compartment, made up of cells that have a very early commitment to a particular developmental fate (Fraser *et al.*, 1990). The neural crest cells that migrate and form the bulk of the facial mesenchyme arise from the same axial level of neural tube as the rhombomeres whose neurones will ultimately innervate that mesenchyme. Neural crest cells destined for the first branchial arch migrate essentially from rhombomeres 1 and 2, whilst those for the second and third arches migrate from rhombomeres 4 and 6, respectively. The even numbered rhombomeres (2, 4, and 6) contain the exit points for cranial nerves V, VII, and IX, nerves that will innervate branchial arches 1, 2, and 3. This leads to the concept that an axial-level specific code exists which is established when the neural crest cells still form part of the neural plate. Cells recognize each other and have a positional identity. Following their migration into the arches, they produce the individual structures that make up the composite head in an orderly and integrated manner.

Clearly, these mechanisms of craniofacial development are under genetic control. How do those genes that are involved produce the complex, regionalized structures that form the building blocks of the developing the head and neck? It is helpful to consider those genes involved in embryogenesis as encoding a set of instructions or rules of assembly. Implementation of these one-dimensional rules, via gene expression and protein interaction, produces the three-dimensional embryo (Thorogood and Ferretti, 1992). In recent years, a number of genes and gene families have been identified that play a critical role in establishing regional identity, including the various components of the vertebrate head. In order to understand these genes completely we have to look at a more humble organism, the fruitfly *Drosophila melanogaster*. Segmental organization of the relatively simple fly embryo gives a number of clues as to how compartmentalization of the rather more complex vertebrate head is achieved.

Homeotic Genes

Construction of the *Drosophila* embryo, larva, and ultimately the adult fly is also based upon segmentation. The basic fly body plan consists of a head, three thoracic segments, eight abdominal segments, and a tail. Once these basic segments have been established, a group of genes known as homeotic genes specify their characteristic structure. Homeotic genes encode transcription factors which act as regulators of downstream gene activity and are characterized by the presence of a highly conserved 180-basepair sequence called the homeobox. The homeobox encodes a 60-amino acid helix-loop-helix DNA binding

motif within the encoded transcription factor. In the fly, homeotic genes are predominantly clustered in two regions (*Antennapedia* and *bithorax*) on chromosome 3, which together make up the single *HOM-C* complex. The axial level of the fly in which these genes function displays a direct linear relationship with their position on the chromosome, a term known as colinearity (Lewis, 1978). Those expressed in the most anterior head end of the fly are found at the furthest (3′ most) end of the chromosome, whilst those in the thorax and abdomen are found progressively further along toward the 5′ end. To put this more simply, the *HOM-C* complex serves as a molecular representation of the anterior-posterior embryonic axis of the developing fly. The homeotic genes along the *HOM-C* complex provide a combinatorial code for the specification of each regional embryonic segment. Mutations in these genes can lead to bizarre homeotic transformations where one segment of the fly can assume the phenotype of another. As an example of the power of these genes, one of them, *Antennapedia* specifies identity of the second thoracic segment, in the dominant mutation of *Antennapedia* this gene becomes expressed inappropriately in the head of the fly. As a result of this, there is a growth of thoracic legs from the head sockets instead of antenna.

Vertebrate Hox *Genes*

In the early 1980s biologists began searching for genes containing the *Drosophila* homeobox in vertebrates, reasoning that the highly conserved nature of the homeobox between homeotic genes might have been preserved during evolution. If this was the case, then these genes might play a key role during vertebrate development. In a landmark evolutionary survey, using DNA from a variety of species, it was shown that the homeobox is not confined to insects, but is also found in vertebrates (McGinnis *et al*., 1984). Considering humans are separated from flies by around 600 million years this conservation was astounding. The first vertebrate homeobox was rapidly cloned in the frog *Xenopus levis* (Carrasco *et al*., 1984) and this was soon followed by the mouse (McGinnis *et al*., 1984). The degree of sequence similarity to the *Drosophila* homeobox was remarkable, confirming that the genetic control of development was more universal than previously imagined. These vertebrate genes were called *Hox* genes, and as more were cloned it became clear that during the course of evolution considerable duplication and divergence had occurred from the original ancestral cluster (Duboule and Dollé, 1989; Graham *et al*., 1989). In the mouse and human genomes there are 39 *Hox* genes related to *Drosophila* homeotic genes. These *Hox* genes are arranged in four clusters (instead of one in the fly) on four different chromosomes; *Hoxa-d* in mice and *HOXA-D* in man (Scott, 1992; Figure 1).

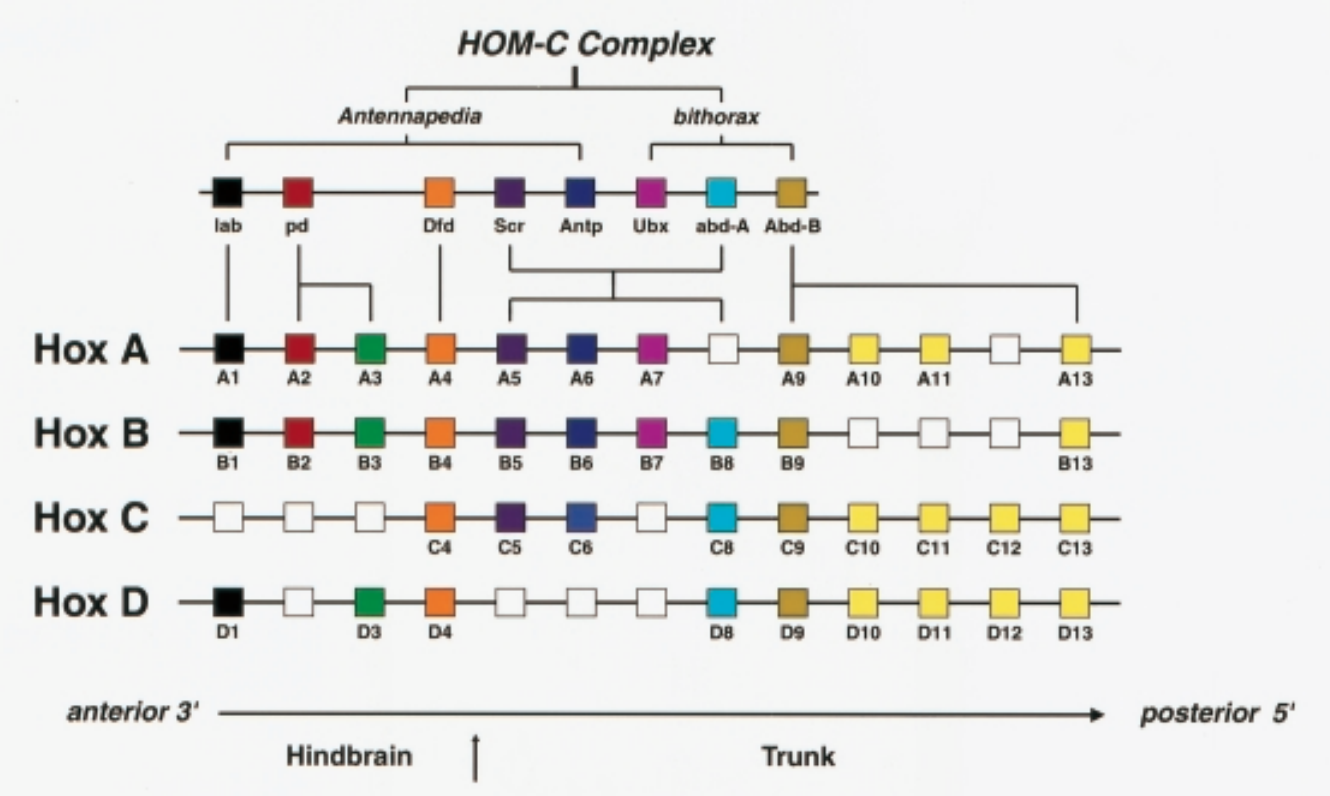

FIG. 1 The 39 human *Hox* genes are organized in four clusters on four chromosomes. They are derived from a single ancestral cluster from which the single *HOM-C* complex in *Drosophila* is also derived. *HOM-C* is composed of two regions: *Antennapedia* (which contains five genes—*labial*, *proboscipedia*, *Deformed*, *Sex combs reduced*, *Antennapedia*) and *bithorax* (containing three genes—*Ultrabithorax*, *abdominal A*, *Abdominal B*). Cluster duplication during evolution has led to the concept of paralogous groups of *Hox* genes. Thus, groups of up to four genes derived from a common ancestral gene in the primitive cluster can be identified based upon sequence homology. The paralogues can exhibit similar expression domains along the anterio-posterior axis of the embryo leading to the concept of functional redundency between genes (diagram based upon Scott, 1992).

A Vertebrate Hox Code

The expression of *Hox* genes in the vertebrate embryo can be seen along the dorsal axis within the CNS, from the anterior region of the hindbrain through the length of the spinal cord. The patterns of expression of these genes show a very precise spatial restriction. Each *Hox* gene is expressed in an overlapping domain along the anterior-posterior axis of the embryo, but each gene has a characteristic segmental limit of expression at its anterior boundary. In the developing head, this spatially restricted expression pattern is seen in the hindbrain with the anterior limits of *Hox* gene expression corresponding to rhombomere boundaries at two-segment intervals. As the neural crest migrates from the rhombomeres into specific branchial arches it retains the particular combination or code of *Hox* gene expression that is characteristic of the rhombomeres from which it originated. Thus, the neural crest from each axial level conveys a unique combinatorial Hox code (Figure 2). This code can be considered to specify form and pattern for the different branchial arch derived regions of the head and neck. It should be noted, however, that the neural crest destined for the first branchial arch, from which the maxillary and mandibular processes develop, does not express *Hox* genes related to the homeotic homeobox (Hunt *et al*., 1991). It is subfamilies of homeobox genes, more diverged from the ancestral *Hox* genes, that are expressed in spatially restricted patterns within the first branchial arch (MacKenzie *et al*., 1992; Sharpe, 1995).

Testing the Hox Code

There is now some experimental evidence to suggest that *Hox* genes are responsible for controlling the mechanisms that result in morphogenesis of regions of the head and neck. One method of testing the Hox code is via the use of transgenic technology, either by disrupting or over-expressing a particular *Hox* gene in transgenic mice. Targeted disruption of the *Hoxa-2* gene, which is normally expressed in the second branchial arch, leads to a loss of some specific second arch structures such as the stapes. In addition to this, there is also a duplication of proximal first arch structures which are fused to the ones that have developed normally (Gendron-Maguire *et al*., 1993; Rijli *et al*., 1993). In other words, this gene deletion has produced a type of homeotic transformation. An absence of *Hoxa-2* leads to cells of the second arch adopting the identity of a first arch. *Hoxa-2* is clearly involved in patterning the second branchial arch and its derivatives. Another *Hox* gene, *Hoxd-4* is normally expressed in the spinal cord, with an anterior limit of expression at the level of C1. If the expression domain of *Hoxd-4* is experimentally extended beyond C1 into the occipital region of the head, the resulting phenotype exhibits transformation of the skull occipital bones into additional cervical vertebrae (Lufkin *et al*., 1992). Of great significance in this experiment is the fact that the original cervical vertebrae display normal characteristics, it is the anterior boundary of expression of these genes that appears to be crucial.

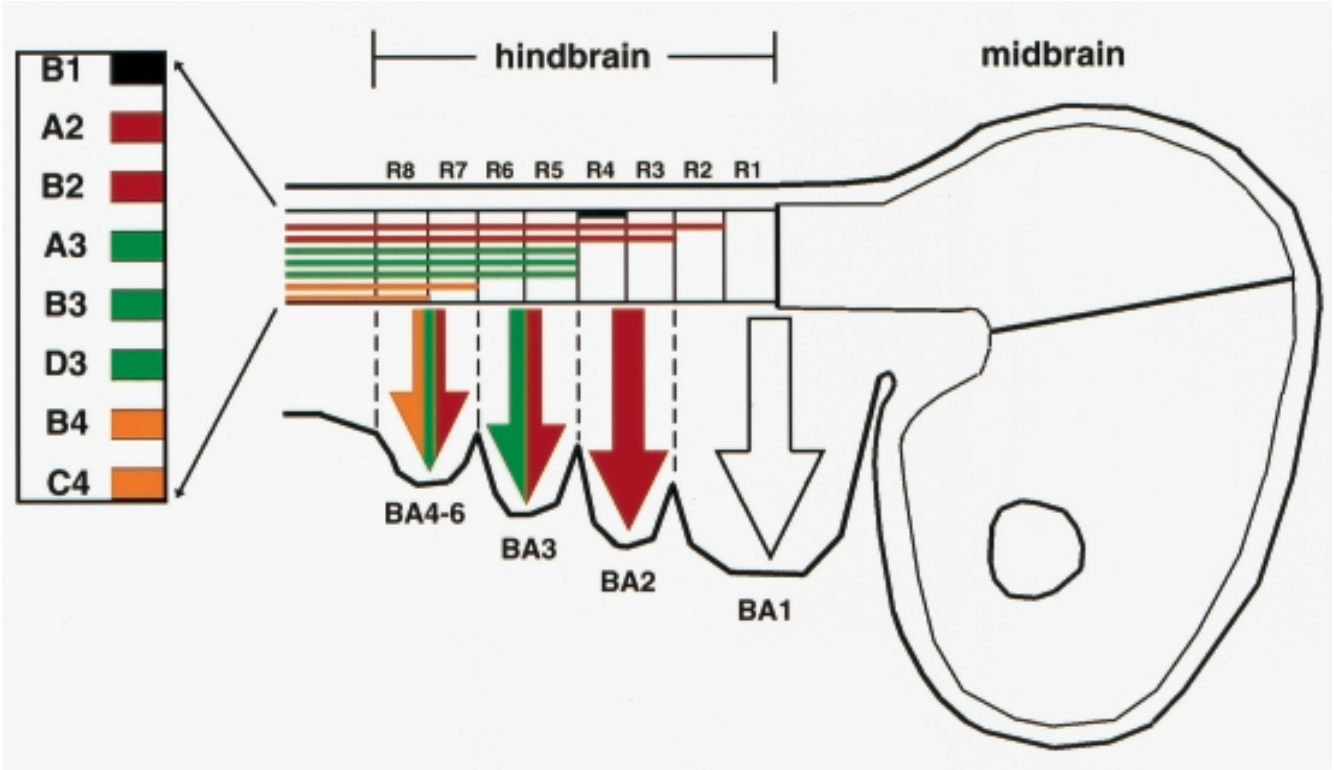

FIG. 2 *Hox* genes are expressed in the migrating neural crest as they are in the rhombomere from which that crest originates. Arch I is populated with crest from the posterior mesencephalon and R1/R2 (with a small contribution from R3), none of these cells express *Hox* genes. Arch II is populated with crest from R4 (with minor contributions from R3 + R5) and expresses *Hoxa-2* (in red). Arch III is populated by crest cells from R6 with minor contributions from R5 + R7, these cells express *Hox* genes from paralogous groups 2 and 3 (i.e. *Hoxa-2*, red, and *Hoxa-3*, green). Arches IV–VI form a poorly individualized group of arches populated essentially by R7 crest, this crest expresses the paralogous *Hoxa-2* (red) and *Hoxa-3* (green), and the orthologue *Hoxb-4* (orange) (diagram based upon Hunt *et al*., 1991).

These results demonstrate a role for *Hox* genes in patterning regions of the developing head. Some transgenic manipulations, however, produce less predictable phenotypes that are more difficult to explain. Targeted disruption of the *Hoxa-3* gene results in abnormalities consistent with its anterior boundary of expression between the second and third arches. However, these mice also have craniofacial defects in tissues derived from the first and second arches, which are anterior to the expression domain of *Hoxa-2* (Chisaka and Capecchi, 1991; Manley and Capecchi, 1995). Functional redundancy between paralogous groups and varying degrees of interaction between *Hox* genes and other regulatory elements does mean that the relationships between these genes can be far from straightforward. There is some concern that the manipulation of a particular *Hox* gene in isolation can affect the normal regulatory mechanisms that govern its expression. The clustered nature of these genes may produce expression patterns that are radically affected when individual genes are altered in isolation (Krumlauf, 1994). These genes are clearly involved in patterning, but their inter-relationships are complex.

Patterning the Upper Head

The expression domains of the classical genes of the Hox cluster do not extend into the first branchial arch or more rostral head regions. *Hox* genes do not, therefore, appear to be involved in specification of neural crest from these more anterior levels. Conclusive evidence for segmentation of the mid- and forebrain regions of the CNS remains elusive. In *Drosophila*, two genes that contain homeobox domains, but which are unrelated to genes of the *HOM-C* locus, are expressed in the anterior regions of the developing head: empty spiracle (*ems*) and orthodenticle (*otd*). The mouse homologues of *ems* and *otd* have been isolated (*Emx-1* and *Emx-2*; *Otx-1* and *Otx-2*), and they also show regional expression in the rostral brain of mouse embryos. Significantly, these expression domains have very specific rostral and caudal boundaries of expression, suggesting a possible genetic code for regional patterning of the brain (Simeone *et al*., 1992; Puelles and Rubenstein, 1993). In the homozygous knockout of *Otx-2*, the mouse fails to develop any head structures anterior to rhombomere 3, indicating an essential function of *Otx-2* in the formation of the rostral head (Matsuo *et al*., 1995; Ang *et al*., 1996). In the heterozygous mutants, craniofacial malformations include a loss of lower jaw structures and the eyes (Matsuo *et al*., 1995). These defects are consistent with human otocephalic mutations (Cohen, 1989) and, interestingly, the affected structures appeared to correspond to the most posterior and anterior domains of *Otx-2* expression, regions where *Otx-1* is not expressed.

The Face and Jaws

A number of other homeobox-containing genes are expressed in the maxillary and mandibular arches, and developing facial primordia. These genes, which all encode homeodomain-containing transcription factors, include *Msx-1*, *Msx-2*, *Dlx1-6*, and *Barx-1*. Again, many of these homeobox-containing genes are related to families of genes found in *Drosophila*. Knockout studies have confirmed that these genes perform essential roles during the formation of the facial complex.

Members of the *Msx* gene family *(Msx-1* and *Msx-2*) are normally expressed strongly in the neural crest derived mesenchyme of the developing facial prominences, and there is now strong evidence for a role of these genes in specification of the skull and face (Ferguson, 2000). Targeted disruption of *Msx-1* in the mouse produces a number of defects in facial structures. There is cleft palate associated with a loss of the palatine shelves in both the maxillary and palatine bones, maxillary and mandibular hypoplasia, and a highly penetrant arrest of tooth formation at the bud stage of development (Satokata and Maas, 1994). *Msx-2* -/- mice have defects in skull ossification with persistence of calvarial foramen. This arises as a result of defective osteoprogenitor proliferation during calvarial morphogenesis (Satokata *et al*., 2000).

Members of the multi-gene *Dlx* family are expressed in a complex pattern within the embryonic ectoderm and mesenchyme of the maxillary and mandibular processes of the first arch (Bulfone *et al*., 1993). Targeted mutations in *Dlx-1*, *Dlx-2*, and *Dlx-1/-2* provide evidence that these genes are required for the development of neural crest derived skeletal elements of the first and second branchial arches (Qiu *et al*., 1997). Analysis of these mutants reveals that *Dlx-1* and *Dlx-2* regulate proximal first arch structures and that, in the mandibular primordium, there is considerable functional redundancy of *Dlx-1* and *Dlx–2* with other members of the *Dlx* family.

Mandibular Phenotypes

Goosecoid is another homeobox-containing transcription factor, originally isolated in *Xenopus* from a dorsal blastopore lip cDNA library. The dorsal blastopore lip has long been known to be ultimately responsible for organization of the complete body axis in the early embryo. However, when *goosecoid* was knocked out in transgenic mice they formed a body axis normally, but exhibited a number of craniofacial defects (Rivera-Pérez *et al*., 1995; Yamada *et al*., 1995). In wild type mice, *goosecoid* transcripts had been detected at later stages of development in the osteogenic mesenchyme of the developing mandible, tongue, and middle ear. In the mutants, the mandible was hypoplastic, and lacked coronoid and angular processes, whilst there were defects in several bones, including the maxillary, palatine, and pterygoid. As a homeobox-containing transcription factor it would appear that *goosecoid* is involved in essential inductive tissue interactions during formation of the head, but has a redundant function in the mouse gastrula organizer. Another gene that has produced an even more perplexing phenotype is *endothelin-1* (*ET-1*). *Endothelin-1* encodes a vasoactive peptide expressed in vascular endothelial cells and is thought to play a role in the regulation of blood pressure. Mice with targeted disruption of *ET-1* have no abnormalities in their cardiovascular system but do have a marked reduction in tongue size, micrognathia and cleft palate (Kurihara *et al*., 1994). Components of the *ET* pathway are now known to be involved in development of the cephalic neural crest. One of the two G protein-coupled endothelin receptors, *ET-A* is expressed

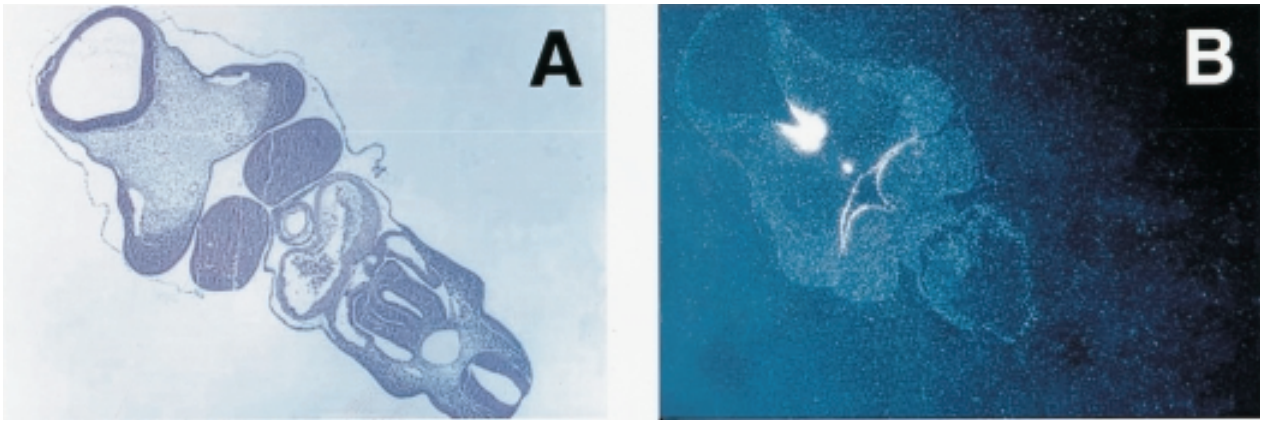

FIG. 3 Midline expression of Sonic hedgehog. Serial coronal sections of a developing E9.5 mouse embryo. (A) Histology (haematoxylin and eosin). (B) 35S radioactive labelled *in situ* hybridization. Note strong expression of Shh in the floorplate of the neural tube, and epithelium of the developing maxillary and mandibular processes.

in the neural crest derived ectomesenchyme of the branchial arches, whilst its primary ligand, ET-1, is expressed in arch epithelium, pharyngeal pouch endothelium, and arch core paraxial mesoderm. The ET-A/ET-1 pathway appears to be important for proper patterning of the caudal regions of the first arch (Tucker *et al*., 1999). Targeted disruption of *ET-A* or *ET-1* in mice produce craniofacial defects that resemble a human condition called CATCH-22, which is characterized by abnormal facies and cardiovascular defects (Wilson *et al*., 1993). It has recently been shown that the craniofacial defects in the *ET-A* -/- mice are, in part, due to an absence of the *goosecoid* transcription factor (Clouthier *et al*., 1998).

Patterning the Midline

Sonic hedgehog (*Shh*) is the vertebrate homologue of the *Drosophila* hedgehog segment polarity gene. In the vertebrate embryo, *Shh* encodes a signalling peptide that is involved in mediating both long- and short-range patterning in a number of well characterized developmental signalling centres (Hammerschmidt *et al*., 1997). Recently, clues about the regulation of craniofacial morphogenesis have come from studies of the *Shh* gene. Mutations of *Shh* in the mouse (Chiang *et al*., 1996) and human (Belloni *et al*., 1996; Roessler *et al*., 1996) leads to profound abnormalities in craniofacial morphogenesis. Loss of Shh produces defective patterning of the neural plate resulting in holoprosencephaly, a failure of cleavage in the midline forebrain, and cyclopia. Later in development *Shh* is expressed in the ectoderm of the fronto-nasal and maxillary processes and has been shown to be essential for their normal development (Wall and Hogan, 1995; Helms *et al*., 1997; Figure 3). By manipulating developing chick embryos, it has recently been shown that a transient loss of Shh signalling in these regions of the developing face can result in defects analogous to hypotelorism and cleft lip/palate, which are characteristic features of the milder forms of holoprosencephaly. In contrast, excess Shh leads to medio-lateral widening of the fronto-nasal process resulting in hypertelorism. In severe cases this can lead to facial duplications (Hu and Helms, 1999).

Conclusions

The study of gene function continues to demonstrate how an understanding of the basic science behind development can lead to advances of direct relevance to the clinician. Many human syndromes and genetic abnormalities have now been attributed to defects in individual genes. It is only by understanding the processes involved during normal development that we can begin to unravel the mechanisms that are responsible when things go wrong. As our knowledge of these processes increases so do the possibilities of utilizing this information for clinical benefit, principally to aid with prenatal diagnosis and ultimately, to allow for the possibility of therapeutic intervention.

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References

Ang, S-L., Ou, J., Rhinn, R., Daigle, N., Stevenson, L. and Rossant, J. (1996)
A targeted mouse Otx-2 mutation leads to severe defects in gastrulation and formation of axial mesoderm and to deletion of rostral brain,
Development, **122,** 243–252.

Belloni, E., Muenke, M., Roessler, E., Traverso, G., Siegel-Bartelt, J., Frumkin, A., Mitchell, H. F., Donis-Keller, H., Helms, C., Hing, A. V., Heng, H. H., Koop, B., Martindale, D., Rommens, J. M., Tsui, L. C. and Scherer, S. W. (1996)
Identification of Sonic hedgehog as a candidate gene in holoprosencephaly,
Nature Genetics, **14,** 353–356.

Bulfone, A., Kim, H-J., Puelles, L., Porteus, M. H., Grippo, J. F. and Rubenstein, J. L. R. (1993)
The mouse *Dlx-2* (*Tes-1*) gene is expressed in spatially restricted domains of the forebrain, face and limbs in mid-gestation mouse embryos,
Mechanisms of Development, **40,** 129–140.

Carrasco, A. E., McGinnis, W., Gehring, W. J. and DeRobertis, E. M. (1984)
Cloning of a *Xenopus levis* gene expressed during early embryogenesis that codes for a peptide region homologous to *Drosophila* homeotic genes,
Cell, **37,** 409–414.

Chai, Y., Jiang, X., Yoshihiro, I., Bringas, P., Han, J., Rowitch, D. H., Soriano, P., McMahon, A. P. and Sucov, H. M. (2000)
Fate of the mammalian neural crest during tooth and mandibular morphogenesis,
Development, **127,** 1671–1679.

Chiang, C., Litingtung, Y., Lee, E., Young, K. E., Corden, J. L., Westphal, H. and Beachy, P. A. (1996)
Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function,
Nature, **383,** 407–413.

Chisaka, O. and Cappecci, M. R. (1991)
Regionally restricted developmental defects reulting from targeted disruption of the mouse homeobox gene *Hox1.5*,
Nature, **350,** 473–479.

Clouthier, D. E., Hosoda, K., Richardson, J. A., Williams, S. C., Yanagisawa, H., Kuwaki, T., Kumada, M., Hammer, R. E. and Yanagisawa, M. (1998)
Cranial and cardiac neural crest defects in endothelin-A receptor deficient mice,
Development, **125,** 813–824.

Cohen, M. M. (1989)
Perspectives on holoprosencephaly. Part I. Epidemiology, genetics, and syndromology,
Teratology, **40,** 211–236.

Couly, G. and Le Douarin, N. (1987)
Mapping of the early neural primordium in quail-chick chimeras. II. The prosencephalic neural plate and neural folds: implications for the genesis of cephalic human congenital abnormalities,
Developmental Biology, **120,** 198–214.

Couly, G. and Le Douarin, N. (1990)
Head morphogenesis in embryonic avian chimeras: evidence for segmental pattern in the ectoderm corresponding to the neuromeres,
Development, **108,** 543–558.

Duboule, D. and Dollé, P. (1989)
The structural and functional organization of the murine HOX gene family resembles that of *Drosophila* homeotic genes,
EMBO, **8,** 1497–1505.

Ferguson, M. W. J. (1993)
Craniofacial morphogenesis and prenatal growth,
In: W. C. Shaw (Ed.) *Orthodontics and Occlusal Management*, Butterworth-Heinemann, Oxford, pp. 1–25.

Ferguson, M. W. J. (2000)
A hole in the head,
Nature Genetics, **24,** 330–331.

Fraser, S., Keynes, R. and Lumsden, A. (1990)
Segmentation in the chick embryo hindbrain is defined by cell lineage restrictions,
Nature, **344,** 431–435.

Gendron-Maguire, M., Mallo, M., Zhang, M. and Gridley, T. (1993)
Hoxa-2 mutant mice exhibit homeotic transformation of skeletal elements derived from cranial neural crest,
Cell, **75,** 1317–1331.

Graham, A., Papalpoplu, N. and Krumlauf, R. (1989)
The murine and *Drosophila* homeobox clusters have common features of organization and expression,
Cell, **57,** 367–378.

Hammerschmidt, M., Brooke, A. and McMahon, A. P. (1997)
The world according to hedgehog,
Trends in Genetics, **13,** 14–21.

Helms, J. A., Kim, C. H., Hu, D., Minkoff, R., Thaller, C. and Eichele, G. (1997)
Sonic hedgehog participates in craniofacial morphogenesis and is down-regulated by teratogenic doses of retinoic acid,
Developmental Biology, **187,** 25–35.

Hu, D. and Helms, J. A. (1999)
The role of sonic hedgehog in normal and abnormal craniofacial development,
Development, **126,** 4873–4884.

Hunt, P., Wilkinson, D. and Krumlauf, R. (1991)
Patterning the vertebrate head: murine *Hox 2* genes mark distinct subpopulations of premigratory and migrating cranial neural crest,
Development, **112,** 43–50.

Krumlauf, R. (1994)
Hox genes in vertebrate development,
Cell, **78,** 191–201.

Kurihara, Y., Kurihara, H., Suzuki, H., Kodama, T., Maemura, K., Nagai, R., Oda, H., Kuwaki, T., Cao, W-H., Kamada, M., Jishage, K., Ouchi, Y., Azumi, S., Toyoda, Y., Ishikawa, T., Kumada, M. and Yazaki, Y. (1994)
Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1,
Nature, **368,** 703–710.

Lewis, E. B. (1978)
A gene complex controlling segmentation in *Drosophila*,
Nature, **276,** 565–570.

Lufkin, T., Mark, M., Hart, C. P., Dolle, P., LeMeur, M. and Chambon, P. (1992)
Homeotic transformation of the occipital bones of the skull by ectopic expression of a homeobox gene,
Nature, **359,** 835–841.

Lumsden, A. and Keynes, R. (1989)
Segmental patterns of neuronal development in the chick hindbrain,
Nature, **337,** 424–428.

MacKenzie, A., Ferguson, M. W. J. and Sharpe, P. T. (1992).
Expression patterns of the homeobox gene, *Hox-8*, in the mouse embryo suggest a role in specifying tooth initiation and shape,
Development, **115,** 403–420.

Manley, N. R. and Capecchi, M. R. (1995)
The role of Hoxa-3 in mouse thymus and thyroid development,
Development, **121,** 1989–2003.

Matsuo, I., Kuratani, S., Kimura, C., Takeda, N. and Aizawa, S. (1995)
Mouse Otx-2 functions in the formation of the rostral head,
Genes and Development, **9,** 2646–2658.

McGinnis, W., Garber, R. L., Wirz, J., Kuroiwa, A. and Gerhing, W. J. (1984)
A homologous protein-coding sequence in *Drosophila* homeotic genes and its conservation in other metazoans,
Cell, **37,** 403–408.

Noden, D. M. (1988)
Interactions and fates of avian craniofacial mesenchyme,
Development, **103** (Supplement), 121–140.

Puelles, L. and Rubenstein, J. L. R. (1993)
Expression patterns of homeobox and other putative regulatory genes in the embryonic mouse forebrain suggest a neuromeric organization,
Trends in Neuroscience, **16,** 472–479.

Qiu, M., Bulfone, A., Ghattas, I., Meneses, J. J., Christensen, L., Sharpe, P. T., Presley, R., Pederson, R. A. and Rubenstein, J. L. R. (1997)
Role of the Dlx homeobox genes in proximodistal patterning of the branchial arches: mutations of Dlx-1 and –2 alter morphogenesis of proximal skeletal and soft tissue structures derived from the first and second arches,
Developmental Biology, **185,** 165–184.

Rijli, F. M., Mark, M., Lakkaraju, S., Dierich, A., Dolle, P. and 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.

Rivera-Pérez, J. A., Mallo, M., Gendron-Maguire, M., Gridley, T. and Behringer, R. R. (1995)
Goosecoid is not an essential component of the mouse gastrula organizer but is required for craniofacial and rib development,
Development, **121,** 3005–3012.

Roessler, E., Belloni, E., Gaudenz, K., Jay, P., Berta, P., Scherer, S. W., Tsui, L. C. and Muenke, M. (1996)
Mutations in the human Sonic hedgehog gene cause holoprosencephaly,
Nature Genetics, **14,** 357–360.

Satokata, I. and Maas, R. (1994)
Msx-1 deficient mice exhibit cleft palate and abnormalities of craniofacial development,
Nature Genetics, **6,** 348–355.

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. and Maas, R. (2000)
Msx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation,
Nature Genetics, **24,** 391–395.

Scott, M. P. (1992)
Vertebrate homeobox gene nomenclature (letter),
Cell, **71,** 551–553.

Sharpe, P. T (1995)
Homeobox genes and orofacial development,
Connective Tissue Research, **32,** 17–25.

Simeone, A. D., Acampora, M., Gulisano, A., Stornaiuolo, A. and Boncinelli, E. (1992)
Nested expression domains of four homeobox genes in developing brain,
Nature, **358,** 687–690.

Thorogood, P. and Ferretti, P. (1992)
Heads and tails: recent advances in craniofacial development,
British Dental Journal, **173,** 301–307.

Tucker, A. S., Yamada, G., Grigoriou, M., Pachnis, V. and Sharpe, P. T. (1999)
Fgf-8 determines rostral-caudal polarity in the first branchial arch,
Development, **126,** 51–61.

Wall, N. A. and Hogan, B. L (1995)
Expression of bone morphogenetic protein-4 (BMP-4), bone morphogenetic protein-7 (BMP-7), fibroblast growth factor-8 (FGF-8) and sonic hedgehog (SHH) during branchial arch development in the chick,
Mechanisms of Development, **53,** 383–392.

Wilson, D. I., Burn, J., Scambler, P. and Goodship, J. (1993)
DiGeorge syndrome: part of CATCH 22,
Journal of Medical Genetics, **30,** 852–856.

Yamada, G., Mansouri, A., Torres, M., Stuart, E. T., Blum, M., Schultz, M., DeRobertis, E. M. and Gruss, P. (1995)
Targeted mutation of the murine goosecoid gene results in craniofacial defects and neonatal death,
Development, **121,** 2917–2922.