Journal of Cellular Biochemistry Supplements 32/33:133–140 (1999)

Mammalian *Dlx* Homeobox Gene Control of Craniofacial and Inner Ear Morphogenesis

Petra Kraus[1] **and Thomas Lufkin**[2]*

[1]Ronald O. Perleman Department of Dermatology, NYU School of Medicine, 550 First Avenue, New York, New York 10016
[2]Brookdale Center for Developmental and Molecular Biology, Mount Sinai School of Medicine, New York, New York 10029

Abstract The *Dlx* homeobox gene family is of ancient origin, with apparent ancestral developmental functions in both nervous system regionalization and appendage (limb) outgrowth. Additional roles in inner ear and craniofacial development were likely acquired by the *Dlx* gene family during the course of animal evolution. Loss-of-function genetic mutations generated in the mouse have revealed a striking role for *Dlx* genes in patterning of the mammalian central nervous system, craniofacial structures and inner ear. Interestingly, none of the individual murine *Dlx* gene mutations to date have resulted in limb defects, suggesting a potentially significant developmental overlap of *Dlx* activity in this embryonic structure. J. Cell. Biochem. Suppls. 32/33:133–140, 1999.

Key words: homeodomain, *distal-less,* embryo, development, mouse mutant

The *Dlx* gene family is an evolutionary-conserved group of homeobox containing transcription factors that, like the *Hox* gene family, plays a fundamental role in the early patterning of embryonic structures. Related to the single *distal-less* (*dll*) gene in *Drosophila melanogaster* [Cohen et al., 1989], in the mouse, six members of the *Dlx* gene family *Dlx1-3* and *Dlx5-7* with homologues in other vertebrates have been described thus far [Stock et al., 1996].

Dlx GENOMIC ORGANIZATION AND EVOLUTIONARY SIGNIFICANCE

The *Dlx* gene family, like many other homeobox-containing gene families, is evolutionarily highly conserved. Although only one *Dlx* gene has been identified in arthropods and amphioxus [Cohen et al., 1989; Holland and Holland, 1996], a pair of *Dlx* genes have been isolated in tunicates [DiGregorio et al., 1995] and six *Dlx* genes arranged in three sets of pairs have been identified in mammals [Stock et al., 1996]. The mammalian *Dlx* genes show a striking genomic organization as pairs of *Dlx* genes with their 3′ ends facing one another, hence in a transcriptionally convergent orientation [Liu et al., 1997; McGuinness et al., 1996; Nakamura et al., 1996; Simeone et al., 1994]. Each pair finds itself in close proximity to a *Hox* gene cluster (Fig. 1). Based on the sequence comparison of the mammalian *Dlx* genes with other vertebrate species, the six *Dlx*-homologue groups can be divided into the clades, *Dlx1/6/7* and *Dlx2/3/5* [Liu et al., 1997; Stock et al., 1996]. This finding is in agreement with the complexity of the extant vertebrate genome resulting from at least two rounds of genome duplication coupled with additional individual gene duplication/deletion events [Ohno, 1993; Sidow, 1996]. An alternative model for the evolution of the tightly linked *Dlx/Hox* gene clusters suggests that a minimum of three rounds of genome duplication events followed by cluster loss events led to the three existing *Dlx/Hox* clusters in vertebrates, with *Hox* clusters A and D expressed in more recently acquired vertebrate structures [Ruddle, 1997].

DLX EXPRESSION PATTERNS DURING EMBRYONIC DEVELOPMENT

Dlx1 and *Dlx2* are expressed in the mesenchyme of the proximal and distal domains of

*Correspondence to: Thomas Lufkin, Brookdale Center for Developmental and Molecular Biology, Mount Sinai School of Medicine, Box 1020, One Gustave L. Levy Place, New York, NY 10029-6574. E-mail: lufkit01@doc.mssm.edu

Received 14 September 1999; Accepted 16 September 1999

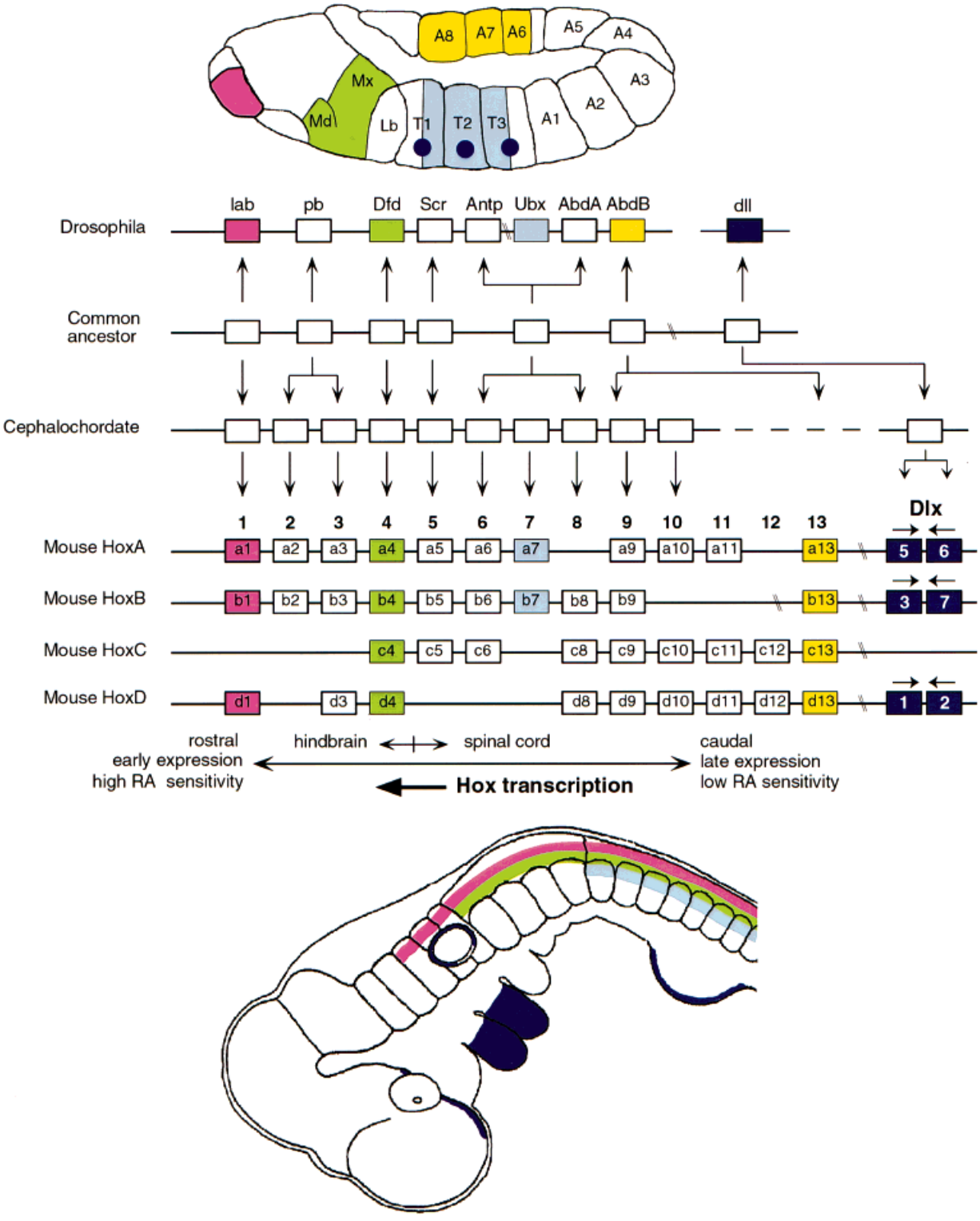

Fig. 1. Diagram of the evolutionary-separated *Dlx/Hox* complexes, chromosomal arrangement and representative expression patterns. **Top:** Diagrammatic representation of a elongated germband-stage *Drosophila* embryo with anterior to the left. **Middle:** Diagrams of the *Dlx/Hox* complexes of *Drosophila,* common ancestor, cephalochordate (amphioxus), and mouse. **Bottom:** Chromosomal position of *Dlx* genes relative to the *Hox* gene clusters. Expression patterns of *Drosophila dll* and mammalian *Dlx5* are shown, along with *Hox* paralogue groups 1, 4, 7, and 13. The expression of paralogue group 13 does not appear in the mammalian embryo as shown, as its expression is too caudally restricted. The collinear arrangement of the *Hox* genes in the complex is indicated relative to spatial expression (rostral versus caudal), timing of expression (early versus late), and retinoic acid (RA) sensitivity (high versus low). The division between *Hox* genes that have anterior borders of expression in the hindbrain or in the spinal cord is shown. The transcriptional orientation of *Dlx* genes is convergent, indicated with an arrow above each mammalian gene. The transcriptional orientation of *Hox* genes is identical, indicated with a bold arrow below the complexes. At bottom, diagrammatic representation of a E10.5 mouse embryo, with anterior to the left. *Dlx/Hox* gene nomenclature is as described [Duboule, 1994].

the first and second branchial arches beginning at E9.5 (Table 1). They show the spatially broadest expression pattern of all studied members of the *Dlx* gene family, with *Dlx2* expressed at higher levels in the first arch ectoderm than is *Dlx1* [Qiu et al., 1997; Robinson and Mahon, 1994; Thomas et al., 1997]. In contrast to *Dlx1* and *Dlx2*, *Dlx3* is only expressed in the skin and distal tips of the branchial arches and is later restricted to the caudal portion of the mandibular process. In contrast to all the other *Dlx* genes, *Dlx3* is not expressed in a detectable manner in the central nervous system (CNS) [Robinson and Mahon, 1994]. Like *Dlx3, Dlx5* and *Dlx6* are also expressed in the distal branchial arch mesenchyme. Expression of *Dlx5* is detected in the distal part of the mandibular region of the first arch at E8.5, and subsequently at E9.5 in the maxillary branch, as well as in the hyoid, and more weakly in the third and fourth branchial arches. *Dlx5* is also expressed in the olfactory and otic placodes, the forebrain, and the neural crest, producing neuroepithelium. Expression in the otic pit was observed as early as E8.0 beginning in the dorsoposterior part of the otic vesicle, followed by expression in the semicircular canals, the endolymphatic duct and the vesicle of the vestibular organ. *Dlx5* is also the first member of the family to be expressed along the rostral and lateral boundary of the neural plate [Acampora et al., 1999; Chen et al., 1996; Depew et al., 1999; Qiu et al., 1997; Simeone et al., 1994; Yang et al., 1998]. Unique to the mammalian *Dlx* family, *Dlx5* and *Dlx6* are expressed in the perichondrial region of all the developing fetal skeletal elements starting as early as cartilage initiation [Acampora et al., 1999; Chen et al., 1996; Simeone et al., 1994] and continuing through the period of mineralization. *Dlx5* and *Dlx6* are expressed in mesenchymal cells that will undergo both endochondral and membranous ossification, and there is in vitro evidence to suggest that *Dlx5* may play a role in osteocalcin expression and osteoblast differentiation [Ryoo et al., 1997]. Expression of *Dlx7* during mouse embryonic development has not been described so far, although its expression was demonstrated by Northern blot analysis in myeloid leukemia cells and other cancer cell lines [Nakamura et al., 1996] (Table 1).

Dlx ROLES IN CRANIOFACIAL AND INNER EAR DEVELOPMENT

With respect to *Dlx* expression during embryonic development in the branchial arches, chondrocranium, and skull vault, it is not surprising that loss of *Dlx* gene activity results in misregulation of craniofacial development. Although ablation of *Dlx* gene function results in a number of phenotypic abnormalities, their influence on craniofacial and inner ear development will be the focus here. The development of craniofacial structures involves complex interactions between epithelial and mesenchymal tissues [reviewed in Hanken and Thorogood, 1993; Le Douarin et al., 1997; Noden, 1988]. The developing cranial anlagen can be subdivided into the neurocranial portion (also referred to as endocranium or primary brain case) encasing the sensory organs: eye, nose, and inner ear as well as the brain; and the splanchnocranium (also referred to as visceral skeleton or branchiocranium), derived from the branchial arches. Both portions of the cranium develop through a combination of membranous and endochondral ossification. In the case of membranous ossification, mesenchymal stem cells differentiate directly into osteoblasts, whereas with endochondral ossification (also termed replacement ossification), the cranial elements are first formed on a cartilaginous framework, subsequently replaced by osteoblasts and mineralized bone. Portions of the skull formed through membranous ossification can also be referred to as dermatocranium, whereas the skull elements that undergo endochondral ossification are also termed the chondrocranium. In the mature skull, bones of membranous or endochondral origin are histologically identical, although their developmental pathways are different. Mammalian cranial sensory organ development begins as ectodermal thickenings adjacent to the rostral neuroepithelium, which give rise to sensory placodes, and subsequently to the olfactory and respiratory epithelium of the nasal cavity, the vestibular and auditory sense organs of the inner ear, and the optic sense organ. In the case of the otic placode, inductive signals from the underlying neuroepithelium are necessary for placode initiation invagination. Subsequent interactions with the surrounding mesenchyme are also necessary for correct morphogenesis and cytodifferentia-

TABLE I. Summary of Genomic Information and Developmental Function of Vertebrate *Dlx* Genes

Gene	Mapping	Homologues in other species	Gene targeting	Embryonic expression	Syndrome in human
Dlx 1 (DIIB)	Mouse: Chr 2 Human: 2q32	Zebrafish (dlx1)	Affects cranial neural crest-derived skeletal elements, not tooth development Dlx1/Dlx2 Maxillary molars missing	E8.5–9: rostral neural plate/tube E9.5: 1st arch, maxillary component, 2nd arch E10: lateral wall of diencephalon E10.5: 1st arch mandibular component, 2nd arch E12.5 on: diencephalon, telencephalon, corpus striatum, hypothalamus, thalamus, forelimb bud P 12 weeks: brain	
Dlx2 (DIIA) (Tes-1)	Mouse: Chr 2 Human: 2q32	Zebrafish (dlx2, 5) *Xenopus* (X-dll1, 4)	Heterozygous normal; homozygous perinatal lethal Altered differentiation of interneurons in the olfactory bulb Affect cranial neural crest-derived skeletal elements, causing cleft palate; tooth development unaffected	E8.5–9: future brain E9.5: 1st arch maxillary component, 2nd arch, limb E10.5: 1st arch, 2nd arch, fore- and hindlimb ectoderm, AER E11.5: hand- and footplate E14.5: thalamus, hypothalamus, diencephalon	
Dlx 3 (DII C)	Mouse: n.d. Human: 17q21.33–22	Xenopus (X-dll2) Newt (NvHBox4) Zebrafish (dlx3)	Early (E9.5) embryonic lethality owing to defects in placental development	E8.5: ectoplacental cone and chorionic plate E9.5 on: 1st arch, 2nd arch E10.5: labyrinthine layer of developing placenta	Tricho-dento-osseus syndrome (MIM 600029)
Dlx 5	Mouse: Chr 6 Human: 7q22	Rat (rdlx) Chicken (dlx5) *Xenopus* (X-dll3) Zebrafish (dlx4)	Homozygous perinatal lethal Craniofacial and inner ear defects Exencephaly in subset of null embryos	E8.5: rostral neural plate and otic placode E9.5 on: 1st arch, 2nd arch E10.5 on: AER E12.5: telencephalon	SHFM1 (MIM 183600)
Dlx 6	Mouse: Chr 6 Human: 7q22	*Xenopus* (Xdll) Zebrafish (dlx6)		E9.5 on: 1st arch, 2nd arch E12.5: telencephalon	
Dlx 7	Mouse: n.d. Human: 17q21.33–22	Zebrafish (dlx 7, 8) Newt (NvHBox5)		Human leukemia cell Mouse mandible	

tion [reviewed in Fekete, 1996; Morsli et al., 1998; Van De Water and Represa, 1991].

Gene targeting of the murine *Dlx1* locus resulted in growth retardation in homozygous newborns that die within the first month of life, owing to a possible dysfunction in the enteric nervous system [Qiu et al., 1997]. By contrast, double knockouts for both *Dlx1/2* die within the first hours of life and show a phenotype similar to that of single knockouts for the *Dlx2* locus [Qiu et al., 1995, 1997]. In addition to defects in neural development [Anderson et al., 1997a,b], craniofacial development is perturbed in the *Dlx1/2* mutants. Although the basisphenoid seemed unaffected, the proximal part of the ala temporalis was missing in the *Dlx1*, *Dlx2*, and *Dlx1/2* mutants. Also, about 30% of the *Dlx2* and *Dlx1/2* mutants showed an ectopic osseous process extending laterocaudally from the basisphenoid. The mutants also showed malformation of the maxillary component of the first branchial arch, with the ventromedial part of the alisphenoid missing and the lateral part malformed in the *Dlx2* and *Dlx1/2* mutants. The incus was also malformed and no longer in contact with the stapes. In addition, a large new cartilage area occurred lateral to the basisphenoid. In all three mutants (*Dlx1*, *Dlx2*, and *Dlx1/2*), the stapes was smaller compared with that of wildtype animals, and the central hole was absent, although with lower penetrance in the *Dlx1* mutants. In addition, the styloids were not connected with the crista parotica of the otic capsule in the *Dlx2* and *Dlx1/2* mutants, and four independent bones resulting in a disruption of the musculoskeletal relationship replaced the squamosal and jugal bones. Furthermore, malformations and misplacement of the maxillary, pterygoid, and palatine bones changed the ventral skull morphology, although the roofing bones (frontal and parietal) appeared normal. The *Dlx1/2* mutants were also affected in their dental development, as they were missing all maxillary molars, which has not been observed in either of the *Dlx1* or *Dlx2* single mutants. A 4-base pair (bp) deletion in human *Dlx3* (resulting in a frameshift) was identified in patients with the tricho-dento-osseus syndrome (MIM 190320), who present with malformations of the teeth and calvaria [Price et al., 1998]. The *Dlx3* gene has been targeted in mice and resulted in embryonic death around day E9.5 of development [Morasso et al., 1999]. The death of the *Dlx3* null embryos appeared to result from placental defects that affected development of the labyrinthine trophoblast layer, an early site of *Dlx3* expression.

Like the *Dlx1* and *Dlx2* null animals, mice with a targeted disruption of *Dlx5* showed severe craniofacial malformations [Acampora et al., 1999; Depew et al., 1999]. As early as E13.5, Meckel's cartilage of the *Dlx5* mutants appeared shorter and was fused to the contralateral partner at a wider angle. At its proximal end, the shaft was dysmorphic, with up to three additional cartilage elements formed. At birth, the craniofacial malformations in the *Dlx5* mice were obvious. The shorter snout likely resulted from a shortening of the posterior part of the maxillary bones, so that no proper connection between the palatine processes of the maxillae and the palatine bones was formed. In severe cases, there was a symmetric aplasia of the nasal capsule and mesethmoid, and the dermal bones that encase the nasal capsule were dysmorphic and small—defects that can be traced to a hypoplasia of the frontonasal process as early as E10.5 in the *Dlx5* null embryos. In addition, the pterygoids were deformed anteriorly, the tympanic ring showed variable reductions in its length, and the alisphenoid was malformed, yet the squamosal and jugal bones appeared normal in the *Dlx5* mutant. The gonial bone was also deformed and occasionally fused to as many as two ectopic bones, and the basioccipital was elongated along the midline. Deformations of the membranous labyrinth probably caused the observed deformation and rotation of the vestibular part of the cartilaginous otic vesicle. Furthermore, the parietal, interparietal, and supraoccipital bones were delayed in their ossification, causing an anterior and posterior open fontanelle and wide cranial sutures. Exencephaly was observed in a subset of the *Dlx5* null animals, coincident with the reduced size of the roofing bones which were either hypomineralized or completely absent. The nasal bones were generally shortened and the nasal epithelium and cartilaginous structures were present either as rudiments or completely absent. In contrast to the absence of maxillary molars in the *Dlx1/2* mutants, the molars in the *Dlx5* null mice were present, but their crowns were poorly mineralized and malformed, as were the incisors.

Dlx5 expression was observed during early otic and olfactory placode formation; however,

their initiation seems to be independent of loss of *Dlx5* expression [Acampora et al., 1999; Depew et al., 1999]. *Dlx5* null embryos did show inner ear defects as early as E10.5, with a failure to form the endolymphatic duct. By E13.5 the dorsal derivatives of the vestibulum were highly affected, lacking the anterior and superior semicircular canals. At later stages (P0), *Dlx5* null mice showed severely affected inner ear development. Overall, the vestibulum was smaller and misshapen, and the horizontal semicircular canal was reduced, while the anterior and posterior semicircular canals were fused into one vesicle. In addition, the cochlea completed only one of its normal 1.5 turns. The chondrocranial otic capsule surrounding the inner ear epithelium was malformed in a manner that paralleled the epithelial defects, possibly indicating a secondary effect. The inner ear epithelium of the *Dlx5*-deficient mice appeared thinner and of a different cell type composition compared with wildtype animals. In addition, the cristae ampullaris were missing in the mutants. Surprisingly, the sacculus was not obviously affected, nor were their sensory epithelia. In addition to the inner ear defects, the otic capsules, bones of the middle ear, and associated soft tissues were affected in the *Dlx5* null mice, and the styloid process appeared shorter than normal and rotated towards the midline.

Dlx5 appears to have a primary role in patterning of craniofacial and inner ear tissues, coupled with a secondary role in osteoblast differentiation as is evidenced by a complete loss of mineralized calvaria in a subset of the null animals. It is likely that the craniofacial malformations in the *Dlx5* knockout mice are also due in part to a defect in osteogenesis, however the axial and appendicular skeleton which also expresses *Dlx5* and *Dlx6,* show no or minor irregularities [Acampora et al., 1999; Depew et al., 1999]. Whether this is the result of functional overlap with *Dlx6* remains to be seen from the combined *Dlx5/6* null mutation. Interestingly, although *Dlx1*, *-2*, *-5*, and *-6* are all expressed in the developing limb, primarily in the apical ectodermal ridge (AER), no limb abnormalities for the *Dlx1*-, *Dlx2*-, *Dlx1/2*-, or *Dlx5*-deficient mice have been observed. Whether this is the result of an overlapping developmental function for these *Dlx* genes, or of superfluous nonfunction expression [Erickson, 1993], will be answered by future analysis of mice carrying compound *Dlx* null alleles. To date, no gene-targeting experiments have been described for either *Dlx6* or *Dlx7*.

Dlx GENES FUNCTION AS TRANSCRIPTION FACTORS

Given the similarity in the DNA-binding homeodomains and the overlapping patterns of expression of the mammalian *Dlx* genes, partial target gene overlap or functional redundancy is likely to be an important factor. The early distally restricted expression of *Dlx3, Dlx5*, and *Dlx6* in the branchial arches compared with *Dlx1* and *Dlx2* might allow for compensation for loss of *Dlx1/2* in the mandibular and distal hyoid arches [Depew et al., 1999]. However, given the fact that the *Dlx1*, *Dlx2*, and *Dlx1/2* loss-of-function phenotypes are different suggests that both genes are not fully redundant with one another. More than one transcript size has been described for *Dlx1* and *Dlx5* [McGuinness et al., 1996; Yang et al., 1998], and it is known that *Dlx1* and *Dlx6* are also transcribed as antisense RNAs [Liu et al., 1997; McGuinness et al., 1996]. Whether these also contribute to the complexity of *Dlx* function and regulation remains to be determined.

Many of the functions of transcription factors are mediated through protein-protein interactions. This is especially true of homeodomain-containing transcription factors [Ferretti et al., 1999; Jacobs et al., 1999; Peltenburg and Murre, 1997; Phelan and Featherstone, 1997; Vigano et al., 1998, and references therein]. *Dlx* proteins have been shown to form dimeric complexes in vitro and in vivo with members of the *Msx* homeobox gene family, inhibiting their transcriptional activities in a reciprocal manner [Zhang et al., 1997]. Furthermore, *Dlx* and *Msx* are expressed in numerous overlapping spatiotemporal domains in the developing embryo, in particular the craniofacial region, and null phenotypes from members of both families of genes have overlapping phenotypes [reviewed in Francis-West et al., 1998]. The homeobox-containing gene *goosecoid* seems to be regulated by *Dlx5*. Its targeted disruption results in similar craniofacial abnormalities as observed in *Dlx5* null mice [Belo et al., 1998; Depew et al., 1999; Rivera-Perez et al., 1995; Yamada et al., 1995]. Another gene that seems to be negatively regulated by *Dlx5* is the osteocalcin promoter, where *Dlx5* acts as a repressor either directly or within a more complex regulatory pathway during osteoblast differentiation

[Ryoo et al., 1997]. Although developmental regulators of osteogenesis (*Osf2*) and inner ear development (*Hmx3/Nkx5.1*) phenocopy the defects in *Dlx5* null animals [Ducy and Karsenty, 1998; Wang et al., 1998], neither appears to be regulated by *Dlx5* [Acampora et al., 1999], suggesting that these developmental transcription factors are either positioned upstream of *Dlx5* or are part of an independent genetic pathway.

Given the similarity in expression patterns and homeodomain sequence, and thus DNA-binding specificity, only a combination of the individual *Dlx* knockouts will give complete insight into *Dlx* gene function during embryogenesis. However, since *Dlx* genes probably act through dimerization with other transcription factors, gain-of-function studies, along with the identification of potential *Dlx* cofactors, will be needed to understand more fully the developmental role of the *Dlx* homeobox gene family.

REFERENCES

Acampora D, Merlo GR, Paleari L, Zerega B, Postiglione MP, Mantero S, Bober E, Barbieri O, Simeone A, Levi G. 1999. Craniofacial, vestibular and bone defects in mice lacking the Distal-less-related gene Dlx5. Development 126:3795–3809.

Anderson SA, Eisenstat DD, Shi L, Rubenstein JL. 1997a. Interneuron migration from basal forebrain to neocortex: dependence on *Dlx* genes. Science 278:474–476.

Anderson SA, Qiu MS, Bulfone A, Eisenstat DD, Meneses J, Pedersen R, Rubenstein JLR. 1997b. Mutations of the homeobox genes *Dlx-1* and *Dlx-2* disrupt the striatal subventricular zone and differentiation of late born striatal neurons. Neuron 19:27–37.

Belo JA, Leyns L, Yamada G, De Robertis EM. 1998. The prechordal midline of the chondrocranium is defective in Goosecoid-1 mouse mutants. Mech Dev 72:15–25.

Chen X, Li X, Wang W, Lufkin T. 1996. *Dlx5* and *Dlx6:* an evolutionary conserved pair of murine homeobox genes expressed in the embryonic skeleton. Ann NY Acad Sci 785:38–47.

Cohen SM, Bröner G, Küttner F, Jürgens G, Jäckle H. 1989. *Distal-less* encodes a homeodomain protein required for limb development in *Drosophila*. Nature 338:432–434.

Depew MJ, Liu JK, Long JE, Presley R, Meneses JJ, Pedersen RA, Rubenstein JL. 1999. Dlx5 regulates regional development of the branchial arches and sensory capsules. Development 126:3831–3846.

Duboule D. 1994. Guidebook to the homeobox genes. Oxford: Sambrook & Tooze/Oxford University Press.

Ducy P, Karsenty G. 1998. Genetic control of cell differentiation in the skeleton. Curr Opin Cell Biol 10:614–619.

Erickson HP. 1993. Gene knockouts of c-*src,* transforming growth factor b1, and tenascin suggest superfluous, nonfunctional expression of proteins. J Cell Biol 120:1079–1081.

Fekete DM. 1996. Cell fate specification in the inner ear. Curr Opin Neurobiol 6:533–541.

Ferretti E, Schulz H, Talarico D, Blasi F, Berthelsen J. 1999. The PBX-regulating protein PREP1 is present in different PBX-complexed forms in mouse. Mech Dev 83: 53–64.

Francis-West P, Ladher R, Barlow A, Graveson A. 1998. Signalling interactions during facial development. Mech Dev 75:3–28.

Hanken J, Thorogood P. 1993. Evolution and development of the vertebrate skull: the role of pattern formation. Trends Ecol Evol 8:9–14.

Holland LZ, Holland ND. 1996. Expression of *AmphiHox-1* and *AmphiPax-1* in amphioxus embryos treated with retinoic acid: insights into evolution and patterning of the chordate nerve cord and pharynx. Development 122: 1829–1838.

Jacobs Y, Schnabel CA, Cleary ML. 1999. Trimeric association of hox and TALE homeodomain proteins mediates hoxb2 hindbrain enhancer activity. Mol Cell Biol 19:5134–5142.

Le Douarin NM, Catala M, Batini C. 1997. Embryonic neural chimeras in the study of vertebrate brain and head development. Int Rev Cytol 175:241–309.

Liu JK, Ghattas I, Liu S, Chen S, Rubenstein JL. 1997. Dlx genes encode DNA-binding proteins that are expressed in an overlapping and sequential pattern during basal ganglia differentiation. Dev Dynam 210:498–512.

McGuinness T, Porteus MH, Smiga S, Bulfone A, Kingsley C, Qiu M, Liu JK, Long JE, Xu D, Rubenstein JL. 1996. Sequence, organization, and transcription of the Dlx-1 and Dlx-2 locus. Genomics 35:473–485.

Morasso MI, Grinberg A, Robinson G, Sargent TD, Mahon KA. 1999. Placental failure in mice lacking the homeobox gene Dlx3. Proc Natl Acad Sci USA 96:162–167.

Morsli H, Choo D, Ryan A, Johnson R, Wu DK. 1998. Development of the mouse inner ear and origin of its sensory organs. J Neurosci 18:3327–3335.

Nakamura S, Stock DW, Wydner KL, Bollekens JA, Takeshita K, Nagai BM, Chiba S, Kitamura T, Freeland TM, Zhao Z, Minowada J, Lawrence JB, Weiss KM, Ruddle FH. 1996. Genomic analysis of a new mammalian distal-less gene: Dlx7. Genomics 38:314–324.

Noden DM. 1988. Interactions and fates of avian craniofacial mesenchyme. Development 103:121–140.

Ohno S. 1993. Patterns in genome evolution. Curr Opin Genet Dev 3:911–914.

Peltenburg LTC, Murre C. 1997. Specific residues in the Pbx homeodomain differentially modulate the DNA-binding activity of Hox and Engrailed proteins. Development 124:1089–1098.

Phelan ML, Featherstone MS. 1997. Distinct HOX N-terminal arm residues are responsible for specificity of DNA recognition by HOX monomers and HOX-PBX heterodimers. J Biol Chem 272:8635–8643.

Price JA, Bowden DW, Wright JT, Pettenati MJ, Hart TC. 1998. Identification of a mutation in DLX3 associated with tricho-dento-osseous (TDO) syndrome. Hum Mol Genet 7:563–569.

Qiu MS, Bulfone A, Martinez S, Meneses JJ, Shimamura K, Pedersen RA, Rubenstein JLR. 1995. Null mutation of Dlx-2 results in abnormal morphogenesis of proximal first and second branchial arch derivatives and abnormal differentiation in the forebrain. Gene Dev 9:2523–2538.

Qiu MS, Bulfone A, Ghattas I, Meneses JJ, Christensen L, Sharpe PT, Presley R, Pedersen RA, Rubenstein JLR. 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.

Rivera-Perez JA, Mallo M, Gendron-Maguire M, Gridley T, Behringer RR. 1995. Goosecoid is not an essential component of the mouse gastrula organizer but is required for craniofacial and rib development. Development 121:3005–3012.

Robinson GW, Mahon KA. 1994. Differential and overlapping expression domains of *Dlx-2* and *Dlx-3* suggest distinct roles for distal-less homeobox genes in craniofacial development. Mech Dev 48:199–215.

Ruddle FH. 1997. Vertebrate genome evolution—the decade ahead. Genomics 46:171–173.

Ryoo HM, Hoffmann HM, Beumer T, Frenkel B, Towler DA, Stein GS, Stein JL, van Wijnen AJ, Lian JB. 1997. Stage-specific expression of Dlx-5 during osteoblast differentiation: involvement in regulation of osteocalcin gene expression. Mol Endocrinol 11:1681–1694.

Sidow A. 1996. Gen(om)e duplications in the evolution of vertebrates. Curr Opin Genet Dev 6:715–722.

Simeone A, Acampora D, Pannese M, D'Esposito M, Stornaiuolo A, Gulisano M, Mallamaci A, Kastury K, Druck T, Huebner K, Boncinelli E. 1994. Cloning and characterization of two new members of the vertebrate *Dlx* family. Proc Natl Acad Sci USA 91:2250–2254.

Stock DW, Ellies DL, Zhao ZY, Ekker M, Ruddle FH, Weiss KM. 1996. The evolution of the vertebrate *Dlx* gene family. Proc Natl Acad Sci USA 93:10858–10863.

Thomas BL, Tucker AS, Qui M, Ferguson CA, Hardcastle Z, Rubenstein JL, Sharpe PT. 1997. Role of Dlx-1 and Dlx-2 genes in patterning of the murine dentition. Development 124:4811–4818.

Van De Water TR, Represa J. 1991. Tissue interactions and growth factors that control development of the inner ear. Neural tube-otic anlage interaction. Ann NY Acad Sci 630:116–128.

Vigano MA, Di Rocco G, Zappavigna V, Mavilio F. 1998. Definition of the transcriptional activation domains of three human HOX proteins depends on the DNA-binding context. Mol Cell Biol 18:6201–6212.

Wang W, VandeWater T, Lufkin T. 1998. Inner ear and maternal reproductive defects in mice lacking the *Hmx3* homeobox gene. Development 125:621–634.

Yamada G, Mansouri A, Torres M, Stuart ET, Blum M, Schultz M, De Robertis EM, Gruss P. 1995. Targeted mutation of the murine goosecoid gene results in craniofacial defects and neonatal death. Development 121:2917–2922.

Yang L, Zhang H, Hu G, Wang H, Abate-Shen C, Shen MM. 1998. An early phase of embryonic Dlx5 expression defines the rostral boundary of the neural plate. J Neurosci 18:8322–8330.

Zhang HL, Hu GH, Wang HY, Sciavolino P, Iler N, Shen MM, Abate-Shen C. 1997. Heterodimerization of Msx and Dlx homeoproteins results in functional antagonism. Mol Cell Biol 17:2920–2932.