Molecular and Cellular Neuroscience **13,** 1–8 (1999)
Article ID mcne.1998.0730, *available online at http://www.idealibrary.com on* IDEAL®

REVIEW

Otx Genes and the Genetic Control of Brain Morphogenesis

Dario Acampora,* Massimo Gulisano,† and Antonio Simeone*

**International Institute of Genetics and Biophysics, CNR, Via G. Marconi, 12, 80125 Naples, Italy; and †Laboratorio di Biologia Molecolare, Dipartimento di Scienze Fisiologiche, Università di Catania, Cittadella Universitaria, Viale A. Doria, 6, 95125 Catania, Italy*

Understanding the genetic mechanisms that control brain patterning in vertebrates represents a major challenge for developmental neurobiology. The cloning of genes likely to be involved in the organization of the brain and an analysis of their roles have revealed insights into the molecular pathways leading to neural induction, tissue specification, and regionalization of the brain. Among these genes, both *Otx1* and *Otx2,* two murine homologs of the *Drosophila orthodenticle* (*otd*) gene, contribute to several steps in brain morphogenesis. Recent findings have demonstrated that *Otx2* plays a major role in gastrulation and in the early specification of the anterior neural plate while *Otx1* is mainly involved in corticogenesis, and *Otx1* and *Otx2* genes cooperate in such a way that a minimal level of OTX proteins are required for proper regionalization and subsequent patterning of the developing brain. Finally, experiments have shown functional equivalence between *Drosophila otd* and vertebrate *Otx* genes, suggesting a surprising conservation of function required in brain development throughout evolution.

The adult brain consists of a number of regions and subregions that are characterized by diverse cell types deriving from a neuroepithelial sheet of cells in the embryo. During brain development, distinct regions in this cell layer are specified following a precise patterning mechanism conferring to different cell types the appropriate regional identity (reviewed in Rubenstein *et al.*, 1998). All the subsequent events, such as outgrowth and axonal pathfinding to form specific connections, depend on the correct prepatterning of the rostral neural plate. In the past decade, several genes involved in brain morphogenesis have been identified, first in *Drosophila* and then in many other species.

These genes encode for signaling molecules and transcription factors involved in the specification of fore-midbrain regions of vertebrates (reviewed in Lumsden and Krumlauf, 1996; Beddington and Robertson, 1998; Rubenstein *et al.*, 1998). *In vivo* inactivation of some of these genes revealed heavy developmental abnormalities resulting from impaired regional specification and / or cell-type induction (reviewed in Bally-Cuif and Wassef, 1995).

Among these, a particular interest derives from the study of *Otx1* and *Otx2* genes, two vertebrate homologs of the *Drosophila orthodenticle* (*otd*) gene (Cohen and Jürgens, 1990; Finkelstein and Perrimon, 1990; Simeone *et al.*, 1992, 1993; Finkelstein and Boncinelli, 1994).

Otx1 Plays a Role in Corticogenesis and Sense Organ Development

During murine embryonic development, *Otx1* begins to be expressed at 8 days postcoitum (d.p.c.) in the neuroectoderm, corresponding to the presumptive fore-midbrain. During mid-late gestation, its expression becomes progressively localized in restricted areas of the fore-midbrain (Simeone *et al.*, 1992, 1993).

During corticogenesis, at midgestation, *Otx1* is expressed in the ventricular zone and in the cortical plate (Simeone *et al.*, 1992; Frantz *et al.*, 1994). At the end of gestation, the *Otx1* signal is weakened in the ventricular zone and, postnatally, it is expressed in a subset of neurons of layers 5 and 6 (Frantz *et al.*, 1994). *Otx1* is also expressed at early stages in precursor structures of sense organs corresponding to the olfactory placode, otic and optic vesicles (Simeone *et al.*, 1993). Later on, *Otx1* is transcribed in the olfactory epithelium, the sacculus, the cochlea, and the ducts of the inner ear as well as in the iris, the ciliary process in the eye and the lachrymal glands primordia (Simeone *et al.*, 1993).

Null mutant mice in which *Otx1* was replaced by the

1044-7431/99 $30.00

lacZ reporter gene suffer from spontaneous epilepsy with both focal and generalized seizures (Acampora *et al.*, 1996). The anatomohystological analysis of homozygous animals revealed an overall reduction of the cerebral cortex, specifically in the perirhinal and temporal areas, where neuronal layers resulted indistinguishable. The absence of sulcus rhinalis and a shrunken hippocampus were also evident. These phenotypes can be correlated to the reduction of cell proliferation within the neuroepithelium of dorsal telencephalon at 9.5 d.p.c. On the contrary, superior and inferior colliculi of the mesencephalon were found to be enlarged. These brain abnormalities could be responsible for generating the epileptic phenotype and demonstrate that the *Otx1* gene product is required for proper brain functions (Acampora *et al.*, 1996). Further morphological defects were found in the acoustic sense organs (lack of the lateral semicircular duct) and the visual sense organs (reduction of the iris, absence of the ciliary process). Lachrymal and Harderian glands were also absent (see Table 1). In addition, *Otx1* null mice show a transient dwarfism and hypogonadism due to a prepubescent stage-specific control of pituitary levels of GH, FSH, and LH (Acampora *et al.*, 1998c).

Otx2 Is Required in Early Gastrulation

At pre-early streak stage, *Otx2* is already transcribed throughout the entire epiblast and in the visceral endoderm (VE). During gastrulation, its expression becomes progressively confined to the anterior third of the embryo, spanning all three germ layers (Simeone *et al.*, 1993; Ang *et al.*, 1994). The neuroectoderm territory expressing *Otx2* at late gastrula stage includes the prosencephalon and mesencephalon. Subsequently, *Otx2* demarcates the mesencephalic side of the mesencephalic / metencephalic boundary (Simeone *et al.*, 1992; Millet *et al.*, 1996), where the isthmic organizer is localized (Bally-Cuif and Wassef, 1995). *Otx2* remains expressed in these regions until late in gestation. This pattern of expression is consistent with what is found in other vertebrates such as chick (Bally-Cuif *et al.*, 1995), Zebrafish (Mercier *et al.*, 1995), and *Xenopus* (Pannese *et al.*, 1995). Later in development, *Otx1* remains expressed in the dorsal telencephalon, but *Otx2* expression disappears completely from this region at 11 d.p.c. (Simeone *et al.*, 1993).

In vivo genetic manipulation experiments performed in *Xenopus* and in mouse have demonstrated a direct role of *Otx2* in rostral CNS specification.

When synthetic *Otx2* RNA was microinjected into *Xenopus*, dramatic morphogenetic changes occurred, including a reduction in size of trunk and tail structures, and the presence of a second cement gland, a structure formed from the anterior-most ectoderm of the frog embryo (Pannese *et al.*, 1995; Blitz and Cho, 1995).

In mice, *Otx2* deletion results in a lethal phenotype. Homozygous null embryos die during early embryogenesis (by 9.5 d.p.c.), lack the anterior neuroectoderm, which gives rise to the forebrain, midbrain, and rostral hindbrain, and show major abnormalities in their body plan (see Table 2) (Acampora *et al.*, 1995; Matsuo *et al.*, 1995; Ang *et al.*, 1996).

Otx2+/− newborns, generated in an appropriate genetic background, reveal variable penetrance of craniofacial and brain malformations resembling the otocephalic phenotype (Matsuo *et al.*, 1995).

TABLE 1

Phenotypic Abnormalities of *Otx1*−/− and otd^1/otd^1 Mutant Mice

Major phenotypes	*Otx1*−/−	otd^1/otd^1
Behavioral phenotypes		
Epilepsy	Yes	Full recovery
Turning behavior	Yes	Slightly recovered
Cerebral cortex		
Cell proliferation	Reduced	Full recovery
Cell number	Reduced	Full recovery
Temporal cortex	Heavily reduced	Full recovery
Perirhinal cortex	Heavily reduced	Full recovery
Cell-layers in temporal and perirhinal cortices	Disorganized	Full recovery
Mesencephalon	Enlarged	Normal in 15% Intermediate in 50%
Cerebellar foliation	Abnormal	Recovery in 10%
Ear (lateral semicircular duct)	Absent	Absent
Eye		
Iris	Reduced	Recovery in 80%
Ciliary process	Absent	Recovery in 80%
Lachrymal and Harderian glands	Absent	Recovery in 34%

Note. Pituitary impairment of *Otx1*−/− mice (Acampora *et al.*, 1998c) is not reported.

Visceral Endoderm and Otx2 Roles in the Establishment of Anterior Patterning

Early specification and patterning of the CNS primordium are controlled during gastrulation by mechanisms involving both vertical signals from axial mesendoderm to the overlying ectoderm and planar signals acting through the ectodermal plane and originating from the organizer (Doniach, 1993; Ruiz i Altaba, 1993, 1994, 1998; Houart *et al.*, 1998; Rubenstein *et al.*, 1998). In this context, the resulting headless phenotype of $Otx2^{-/-}$ mutant mice might be due to the abnormal development of the prechordal axial mesendoderm, which lacks head organizer activities. Consistent with this hypothesis, previous results from experiments on tissue recom-

TABLE 2
Phenotypic Abnormalities of *Otx2*+/− and *Otx2*−/− Mutant Mice

Major phenotypes	*Otx2*+/−	*Otx2*−/−
Embryo lethality	0–38.7%[a]	100%
Head abnormalities (otocephaly)	84%[b]	
Heart		Abnormal
Body plan		Abnormal
Anterior neuroectoderm		Absent
Visceral endoderm		Not anteriorized
Anterior mesendoderm and node		Absent or strongly impaired
Primitive streak		Abnormal

[a]Frequency of embryo lethality is dependent on the parental genotype (Matsuo *et al.*, 1995).

[b]The otocephalic phenotype includes microphthalmia, micrognathia, anophthalmia, ethmocephaly, agnathia, short nose, and acephaly (Matsuo *et al.*, 1995).

bination indicated that positive signals from the anterior mesendoderm are required to stabilize the ectodermal expression of *En* and *Otx2* genes (Ang and Rossant, 1993; Ang *et al.*, 1994).

However, there is increasing evidence that the anterior visceral endoderm (AVE) in mouse, and the leading edge of the involuting endoderm in *Xenopus*, play a fundamental role in the specification of the anterior neural plate (reviewed in Beddington and Robertson, 1998). In fact, it has been shown that (i) removal of a patch of AVE cells expressing *Hesx1* prevents its subsequent expression in the rostral headfolds, which are reduced and abnormally patterned (Thomas and Beddington, 1996; Dattani *et al.*, 1998); (ii) chimaeric embryos composed of wild-type epiblast and *nodal*−/− VE cells resulted affected in rostral CNS development (Varlet *et al.*, 1997); (iii) microinjection of *cerberus* mRNA—which encodes a secreted factor expressed in the leading edge of the involuting endoderm of *Xenopus* embryo—induces the formation of ectopic head-like structures without a secondary axis (Bouwmeester *et al.*, 1996); (iv) heterotopic transplantation of the node (the mouse organizer) can generate a secondary axis lacking anteriormost neural tissues (Beddington, 1994; Beddington and Robertson, 1998); (v) *HNF-3*$\beta^{-/-}$ embryos, having nonrecognizable node and axial mesendoderm, show an almost normal anterior pattern (Ang and Rossant, 1994), and finally, (vi) the finding that most of the genes expressed in the node or axial mesendoderm cells are also expressed in the AVE at earlier stages suggests that they may overlap in the earliest genetic pathway involved in organizing the head (Thomas and Beddington, 1996; Belo *et al.*, 1997; Ruiz i Altaba, 1998).

Together, this evidence suggests that, at least in the mouse, the organizer might be split into at least two regions, the AVE and the node, which operate at different stages to specify and maintain head and trunk structures, respectively (Beddington and Robertson, 1998).

The lack of the rostral brain in *Otx2*−/− embryos could be due to abnormalities either in tissues having inducing properties, such as AVE (Thomas and Beddington, 1996; Varlet *et al.*, 1997) and prechordal mesendoderm (Lemaire and Kodjabachian, 1996), or in the responding epiblast and anterior neuroectoderm. However, in homozygous embryos in which *Otx2* is replaced by a *lacZ* reporter gene (Acampora *et al.*, 1995), the first abnormality is detected at pre-early streak stage. In fact at this stage, in *Otx2*+/− embryos, *lacZ* is transcribed in both VE and epiblast, while in *Otx2*−/− embryos, *lacZ* transcripts were detected only in the VE. These data indicate that, since *Otx2* is transcribed as early as at the preimplantation stages, maintenance of its transcription in the epiblast requires at least one *Otx2* normal allele in the VE, where *Otx2* is not necessary for its own transcription.

The relevance of *Otx2* in the anterior visceral endoderm has also been demonstrated by the analysis of chimaeric embryos containing *Otx2*−/− epiblast cells and wild-type VE or vice versa (Rhinn *et al.*, 1998). Rescue of the anterior neural plate induction was observed only when wild-type VE was present.

Otx Genes in Brain Patterning

It has been proposed that organizing centers are generated at the boundary between differently specified juxtaposed territories where cooperative interactions result in the production of signaling molecules with inducing properties (Meinhardt, 1983; Ingham and Martinez Arias, 1992; Perrimon, 1994). During development, the morphogenetic fate of distinct brain areas largely relies on specific differentiating programs depending on the interaction between territorial competence and inductive signals produced by organizing centers (reviewed in Rubenstein *et al.*, 1998). Several boundary zones in the developing brain, including the mid/hindbrain junction or isthmus, the zona limitans intrathalamica (ZLI), and the anterior neural ridge (ANR), have been indicated as possible organizing centers and/or barriers to the transmission of patterning signals (reviewed in Rubenstein *et al.*, 1998, and Rubenstein and Beachy, 1998). Genes such as *Fgf8* and *Shh*, expressed in the isthmus and in the ZLI, respectively (Echelard *et al.*, 1993; Crossley and Martin, 1995), encode secreted molecules known to play an inductive role. Evidence from expression analysis (Simeone *et al.*, 1992, 1993), transplantation experiments in the chick (Millet *et al.*, 1996), retinoic acid-induced phenocopies (Simeone *et al.*, 1995; Avvantaggiato *et al.*,

1996), and the finding that, in *Drosophila*, different *OTD* levels are required for the development of specific subdomains of the head (Royet and Finkelstein, 1995) suggest a role for *Otx* genes in patterning the fore-midbrain territories. Thus, in order to test the possibility that an appropriate threshold of OTX proteins (Hirth *et al.*, 1995; Thor, 1995) is required for the mechanisms underlying the regionalization and patterning of the rostral neural tube, the *Otx* gene dosage was altered *in vivo*.

Interestingly, only *Otx1*−/−; *Otx2*+/− embryos showed 100% of macroscopic brain malformations. The presence of an additional functional copy either of *Otx2* (*Otx1*−/−; *Otx2*+/+) or *Otx1* (*Otx1*+/−; *Otx2*+/−) completely recovered the abnormal phenotype, thus indicating that a critical threshold of *Otx* gene product is required for correct brain morphogenesis and that *Otx1* and *Otx2* may cooperate in specifying correct brain patterning (Acampora *et al.*, 1997).

The analysis of the *Otx1*−/−; *Otx2*+/− brains likely results as the consequence of a repatterning process (Fig. 1) involving the anterior displacement of an isthmic-like structure in the caudal diencephalon, the telencephalic acquisition of mesencephalic molecular features, and a more complete transformation of both caudal diencephalon (prosomeres 1 and 2) and mesencephalon into an enlarged metencephalon (cerebellum and pons). Suda *et al.* (1996) presented a similar genetic analysis in a different genetic background, describing phenotypic impairments in *Otx1*+/−; *Otx2*+/− mice.

Together these findings support the existence of a genetic control of brain patterning depending on a precise threshold of OTX proteins that is strictly required to properly specify adjacent territories with different fates, such as the mesencephalon and the metencephalon, and for allowing the correct positioning of the *Fgf8*-inducing properties at the isthmic organizer (Acampora *et al.*, 1997).

otd-Otx Functional Equivalence

Despite the obvious difference in brain anatomy, *Drosophila otd* and mouse *Otx* genes share similarities in their homeodomain, patterns of expression, dose-dependent mechanisms of action of their gene products, and mutant phenotypes. In mutant flies lacking *otd* function, the protocerebral anlage is deleted and some deuterocerebral neuroblasts do not form, giving rise to a dramatically reduced brain (Hirth *et al.*, 1995; Younossi-Hartenstein *et al.*, 1997). Other defects are also observed in the ventral nerve cord and in nonneural structures (Finkelstein *et al.*, 1990). Flies that are homozygotes for

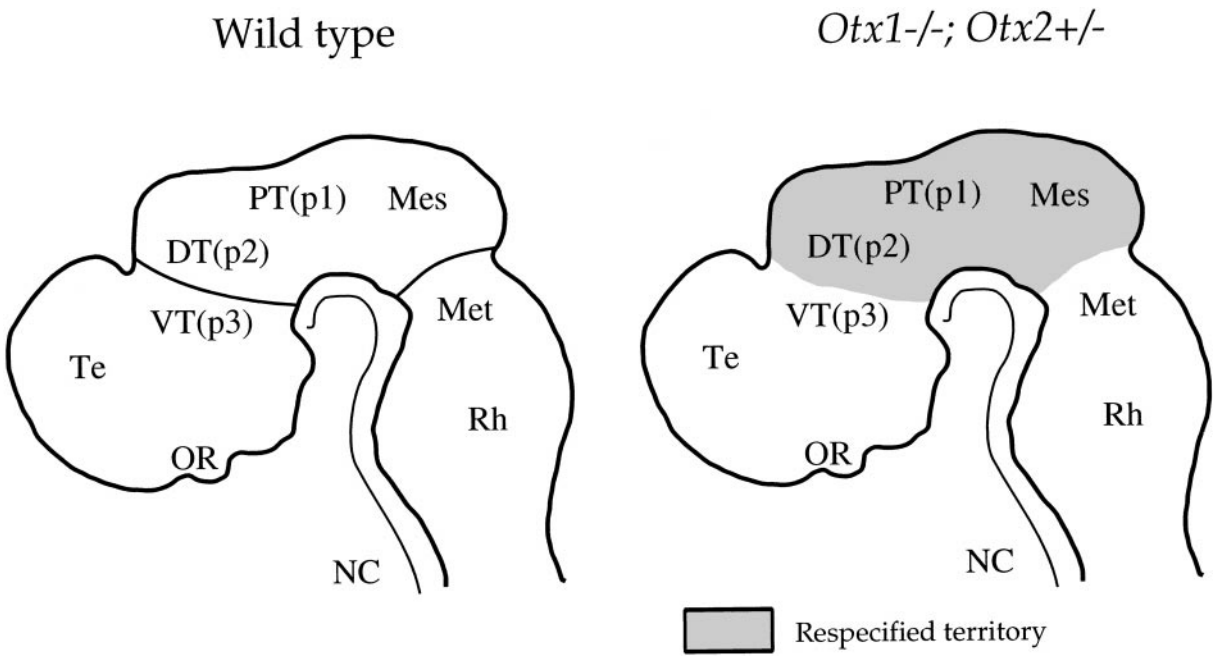

FIG. 1. Schematic representation of brain phenotype at 10.5 d.p.c. in *Otx1*−/−; *Otx2*+/− mutant mice (right) compared to wild type (left). Regions corresponding to mesencephalon, pretectum (p1), and dorsal thalamus (p2) (shaded in grey) are respecified with a posterior identity and isthmus is anteriorly displaced. Abbreviations: DT, dorsal thalamus; Mes, mesencephalon; NC, notochord; OR, optic recess; PT, pretectum; Rh, rhomboencephalon; VT, ventral thalamus; p1, p2, p3, prosomeres 1, 2, and 3.

Ocelliless (*oc*) a different *otd* allele are viable and lack the ocelli (light sensing organs) and associated sensory bristles of the vertex. Moreover, different levels of OTD protein are required for the formation of specific subdomains of the adult head (Royet and Finkelstein, 1995).

In mouse, *Otx* genes are required in early specification and patterning of anterior neuroectoderm, in neuroblast proliferation and corticogenesis, and in visual and acoustic sense organ development (Acampora *et al.*, 1995, 1996, 1997; Matsuo *et al.*, 1995; Ang *et al.*, 1996).

To test whether these similarities among members of the *otd/Otx* gene family may underlie a basic mechanism of action conserved throughout evolution, the murine *Otx1* gene has been replaced with the *Drosophila otd* gene (otd^1/otd^1 mice; Acampora *et al.*, 1998a) and human *Otx* genes have been introduced in *Drosophila otd* null mutants (Leuzinger *et al.*, 1998).

Interestingly, many of the abnormalities of $Otx1-/-$ mice, such as impaired cell proliferation, corticogenesis, and epilepsy are fully rescued by *otd* (Acampora *et al.*, 1998a) regardless of a lower level of OTD (about 30% less) in otd^1/otd^1 mice compared to the OTX1 level in wild-type animals. To a lesser extent, $Otx1-/-$ eye defects and brain patterning alterations detected in $Otx1-/-$; $Otx2+/-$ embryos are also recovered. In contrast, the lateral semicircular canal of the inner ear of $Otx1-/-$ mice is never restored (Table 1).

In similar experiments in the fly, overexpression of human *Otx1* and *Otx2* genes rescues the brain and ventral nerve cord phenotypes of *otd* mutants (Leuzinger *et al.*, 1998) as well as the cephalic defects of adult flies carrying the *ocelliless* mutation (Nagao *et al.*, 1998). Moreover, ubiquitous overexpression of *Otx1* and *Otx2* genes in a *Drosophila* wild-type background is able to induce ectopic neural structures (Leuzinger *et al.*, 1998).

The similarity in *otd/Otx* function is surprising not only because of the different anatomy and complexity of insect and mammalian brains, but also because of the very limited region of homology shared by the proteins and restricted essentially to the homeodomain. These two observations imply that *otd*/*Otx* genes can trigger a basic program of cefalic development through conserved genetic interactions possibly involving a homeobox-mediated choice of the same target sequence and, probably, the same target genes (Sharman and Brand, 1998). On the other hand, the incomplete rescue of either acoustic defect in otd^1/otd^1 mice or brain patterning abnormalities in otd^1/otd^1; $Otx2+/-$ embryos by *Drosophila otd* gene may reflect both quantitative (higher level of *otd* expression) and qualitative (*Otx*-specific) requirements. In particular, failure in recovering the lateral semicircular duct of the inner ear in otd^1/otd^1 mice (Acampora *et al.*, 1998a; Sharman and Brand, 1998) suggests an *Otx1*-specific function acquired during evolution.

Taken together, our data argue in favor of an extended evolutionary conservation between the murine *Otx1* and the *Drosophila otd* genes and support the hypothesis that genetic functions required in mammalian brain development evolved in a primitive ancestor of flies and mice more than 500 million years ago (Wray *et al.*, 1996).

Redundant and Specific Functions between Otx1 and Otx2

Mammalian OTX1 and OTX2 proteins share extensive similarities in their sequences, even though downstream of the *Otx1* homeodomain, the regions of homology to OTX2 are separated by stretches of additional amino acids (Simeone *et al.*, 1993). In order to determine whether $Otx1-/-$ and $Otx2-/-$ divergent phenotypes could derive from differences in the temporal expression or biochemical activity of OTX1 and OTX2 proteins, we have generated mice in which the *Otx2* gene was replaced by a human *Otx1* (*hOtx1*) full-coding cDNA ($hOtx1^2/hOtx1^2$) (Acampora *et al.*, 1998b) or the *Otx1* gene was replaced by a human *Otx2* (*hOtx2*) full-coding cDNA ($hOtx2^1/hOtx2^1$) (Acampora *et al.*, unpublished data).

In homozygous knock-in ($hOtx1^2/hOtx1^2$) mutant embryos, the OTX1 protein is detected only in the VE. This VE-restricted hOTX1 protein recovered gastrulation defects and induction of an early anterior neural plate. However, from 8.5 d.p.c. onwards, $hOtx1^2/hOtx1^2$ embryos fail to maintain fore-midbrain identities, and at the end of gestation, display a headless phenotype in which the body plan shows no detectable defects (Acampora *et al.*, 1998b).

These results indicate that in the VE, *Otx1* and *Otx2* are functionally equivalent, since in this tissue *hOtx1* is sufficient to recover the *Otx2*-requirement for specification of the early anterior neural plate and proper organization of the primitive streak. Moreover, these data provide strong evidence that *Otx2* is necessary in the mesendoderm and/or the neuroectoderm at the late gastrulation stage for the maintenance of anterior patterning of the neural plate. In this context, our findings lead to the intriguing hypothesis that the ability of *Otx2* to be translated in epiblast-like cells might have been established in early vertebrate evolution by acquiring posttranscriptional regulatory elements. This kind of control acquired in neuronal progenitors might have represented a crucial event required for maintenance of fore-midbrain territories and positioning of their boundaries in higher vertebrates.

Homozygous mice in which *Otx1* was replaced with the human *Otx2* cDNA ($hOtx2^1/hOtx2^1$) recover from

epilepsy and corticogenic abnormalities and show a significant improvement in mesencephalon, eye, and lachrymal gland abnormalities. Interestingly, the rescue observed is comparable with that of mice in which *Otx1* is replaced with *otd* (Acampora *et al.*, 1998a).

These data indicate that contrasting phenotypes in *Otx1* and *Otx2* null mice originate mostly from their divergent expression patterns. Interestingly, neither *hOtx2* nor *otd* are able to recover the lateral semicircular duct of the inner ear, suggesting this might be a newly acquired *Otx1*-specific function whose appearance (from gnathostomes onwards) might suggest when *Otx1* functions were established in evolution.

Conclusions and Perspectives

Recent studies have provided fundamental clues on the mysteries surrounding the brain and its development. In this context, *Otx* genes seem to play a crucial role at different levels, both in early phases of neural induction and patterning (*Otx2*) and in later phases of neuronal terminal differentiation (*Otx1*). The *otd-Otx2* or *Otx1-Otx2* genetic cooperation in double mutants (Acampora *et al.*, 1997, 1998a) and the rescue of many of the null phenotypes observed in our knock-in models (Acampora *et al.*, 1998a, 1998b; Acampora *et al.*, unpublished observations) and in *Drosophila* (Leuzinger *et al.*, 1998; Nagao *et al.*, 1998) also indicate the functional conservation existing among these genes.

Future experiments will define *Otx1* and *Otx2* regulatory controls, functional domains of their gene products, as well as molecular partner(s) in order to understand the *Otx* involvement in the developmental pathways that have been created and selected during evolution to specify the greater complexity of the mammalian brain.

ACKNOWLEDGMENTS

We thank A. Secondulfo for skillful secretarial assistance. This work was supported by grants from the EC BIOTECH Programme (to A.S. and M.G.), the Italian Telethon Programme, the Italian Association for Cancer Research (AIRC) and Progetto Finalizzato Biotecnologie to A.S., and a NATO Collaborative Research Grant. M.G. was the recipient of a EU fellowship in the laboratory of A. Lumsden at UMDS, London.

REFERENCES

Acampora, D., Mazan, S., Lallemand, Y., Avantaggiato, V., Maury, M., Simeone, S., and Brûlet, P. (1995). Forebrain and midbrain regions are deleted in *Otx2*−/− mutants due to a defective anterior neuroectoderm specification during gastrulation. *Development* **121:** 3279–3290.

Acampora, D., Mazan, S., Avantaggiato, V., Barone, P., Tuorto, F., Lallemand, Y., Brûlet, P., and Simeone, A. (1996). Epilepsy and brain abnormalities in mice lacking *Otx1* gene. *Nature Genet.* **14:** 218–222.

Acampora, D., Avantaggiato, V., Tuorto, F., and Simeone, A. (1997). Genetic control of brain morphogenesis through *Otx* gene dosage requirement. *Development* **124:** 3639–3650.

Acampora, D., Avantaggiato, V., Tuorto, F.,Barone, P., Reichert, H., Finkelstein, R., and Simeone, A. (1998a). Murine *Otx1* and *Drosophila otd* genes share conserved genetic functions required in invertebrate and vertebrate brain development. *Development* **125:** 1691–1702.

Acampora, D., Avantaggiato, V., Tuorto, F., Briata, P., Corte, G., and Simeone, A. (1998b). Visceral endoderm-restricted translation of *Otx1* mediates recovering of *Otx2* requirements for specification of anterior neural plate and proper gastrulation. *Development* **125:** 5091–5104.

Acampora, D., Mazan, S., Tuorto, F., Avantaggiato, V., Tremblay, J. J., Lazzaro, D., di Carlo, A., Mariano, A., Macchia, P. E., Corte, G., Macchia, V., Drouin, J., Brûlet, P., and Simeone, A. (1998c). Transient dwarfism and hypogonadism in mice lacking *Otx1* reveal prepubescent stage-specific control of pituitary levels of GH, FSH and LH. *Development* **125:** 1061–1072.

Ang, S.-L., and Rossant, J. (1993). Anterior mesendoderm induces mouse *Engrailed* genes in explant cultures. *Development* **118:** 139–149.

Ang, S.-L., Conlon, R. A., Jin, O., and Rossant, J. (1994). Positive and negative signals from mesoderm regulate the expression of mouse *Otx2* in ectoderm explants. *Development* **120:** 2979–2989.

Ang, S.-L., and Rossant, J. (1994). *HNF-3*β is essential for node and notochord formation in mouse development. *Cell* **78:** 561–574.

Ang, S.-L., Jin, O., Rhinn, M., Daigle, N., Stevenson, L., and Rossant, J. (1996). Targeted mouse *Otx2* mutation leads to severe defects in gastrulation and formation of axial mesoderm and to deletion of rostral brain. *Development* **122:** 243–252.

Avantaggiato, V., Acampora, D., Tuorto, F., and Simeone, A. (1996). Retinoic acid induces stage-specific repatterning of the rostral central nervous system. *Dev. Biol.* **175:** 347–357.

Bally-Cuif, L., Gulisano, M., Broccoli, V., and Boncinelli, E. (1995). c-*otx2* is expressed in two different phases of gastrulation and is sensitive to retinoic acid treatment in chick embryo. *Mech. Dev.* **49:** 49–63.

Bally-Cuif, L., and Wassef, M. (1995). Determination events in the nervous system of the vertebrate embryo. *Curr. Opin. Genet. Dev.* **5:** 450–458.

Beddington, R. S. P. (1994). Induction of a second neural axis by the mouse node. *Development* **120:** 613–620.

Beddington, R. S. P., and Robertson, E. J. (1998). Anterior patterning in mouse. *Trends Genet.* **14:** 277–283.

Belo, J. A., Bouwmeester, T., Leyns, L., Kertesz, N., Gallo, M., Gollettie, M., and De Robertis, E. M. (1997). *Cerberus-like* is a secreted factor with neuralizing activity expressed in the anterior primitive endoderm of the mouse gastrula. *Mech. Dev.* **68:** 45–57.

Blitz, I. L., and Cho, K. W. Y. (1995). Anterior neuroectoderm is progressively induced during gastrulation: The role of the *Xenopus* homeobox gene *orthodenticle. Development* **121:** 993–1004.

Bouwmeester, T., Kim, S. H., Sasai, Y., Lu, B., and De Robertis, E. M. (1996). *Cerberus* is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. *Nature* **382:** 595–601.

Cohen, S. M., and Jürgens, G. (1990). Mediation of *Drosophila* head development of gap-like segmentation genes. *Nature* **346:** 482–488.

Crossley, P. H., and Martin, G. R. (1995). The mouse *Fgf8* gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryos. *Development* **121:** 439–451.

Dattani, M. T., Martinez-Barbera, J.-P., Thomas, P. Q., Brickman, J. M., Gupta, R., Mårtensson, I.-L., Toresson, H., Fox, M., Wales, J. K. H.,

Hindmarsh, P. C., Krauss, S., Beddington, R. S. P., and Robinson, I. C. A. F. (1998). Mutations in the homeobox gene *HESX1/Hesx1* associated with septo-optic dysplasia in human and mouse. *Nat. Genet*. **19:** 125–133.

Doniach, T. (1993). Planar and vertical induction of anteroposterior pattern during the development of the amphibian central nervous system. *J. Neurobiol*. **24:** 1256–1276.

Echelard, Y., Epstein, D. J., St-Jacques, B., Shen, L., Mohler, J., McMahon, J. A., and McMahon, A. P. (1993). *Sonic hedgehog*, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* **75:** 1417–1430.

Finkelstein, R., and Perrimon, N. (1990). The *orthodenticle* gene is regulated by *bicoid* and *torso* and specifies *Drosophila* head development. *Nature* **346:** 485–488.

Finkelstein, R., Smouse, D., Capaci, T. M., Spradling, A. C., and Perrimon, N. (1990). The *orthodenticle* gene encodes a novel homeodmain protein involved in the development the *Drosophila* nervous system and ocellar visual structures. *Genes Dev*. **4:** 1516–1527.

Finkelstein, R., and Boncinelli, E. (1994). From fly head to mammalian forebrain: The story of *otd* and *Otx*. *Trends Genet*. **10:** 310–315.

Frantz, G. D., Weimann, J. M., Levin, M. E., and McConnell, S. K. (1994). *Otx1* and *Otx2* define layers and regions in developing cerebral cortex and cerebellum. *J. Neurosci*. **14:** 5725–5740.

Hirth, F., Therianos, S., Loop, T., Gehring, W. J., Reichert, H., and Furukubo-Tokunaga, K. (1995). Developmental defects in brain segmentation caused by mutations of the homeobox gene *orthodenticle* and *empty spiracles* in Drosophila. *Neuron* **15:** 1–20.

Houart, C., Westerfield, M., and Wilson, S. W. (1998). A small population of anterior cells patterns the forebrain during zebrafish gastrulation. *Nature* **391:** 788–792.

Ingham, P. W., and Martinez Arias, A. (1992). Boundaries and fields in early embryos. *Cell* **68:** 221–235.

Lemaire, P., and Kodjabachian, L. (1996). The vertebrate organizer: Structure and molecules. *Trends Genet*. **12:** 525–531.

Leuzinger, S., Hirth, F., Gerlich, D., Acampora, D., Simeone, A., Gehring, W., Finkelstein, R., Furukubo-Tokunaga, K., and Reichert, H. (1998). Equivalence of the fly *orthodenticle* gene and the human *OTX* genes in embryonic brain development of Drosophila. *Development* **125:** 1703–1710.

Lumsden, A., and Krumlauf, R. (1996). Patterning the vertebrate neuraxis. *Science* **274:** 1109–1115.

Matsuo, I., Kuratani, S., Kimura, C., Takeda, N., and Aizawa, S. (1995). Mouse *Otx2* functions in the formation and patterning of rostral head. *Genes Dev*. **9:** 2646–2658.

Meinhardt, H. (1983). Cell determination boundaries as organizing regions for secondary embryonic fields. *Dev. Biol*. **96:** 375–385.

Mercier, P., Simeone, A., Cotelli, F., and Boncinelli, E. (1995). Expression pattern of two *Otx* genes suggests a role in specifying anterior body structures in zebrafish. *Int. J. Dev. Biol*. **39:** 559–573.

Millet, S., Bloch-Gallego, E., Simeone, A., and Alvarado-Mallart, R.-M. (1996). The caudal limit of *Otx2* gene expression as a marker of the midbrain/hindbrain boundary: A study using a chick-*Otx2* riboprobe and chick/quail homotopic grafts. *Development* **122:** 3785–3797.

Nagao, T., Leuzinger, S., Acampora, D., Simeone, A., Finkelstein, R., Reichert, H., and Furukubo-Tokunaga, K. (1998). Developmental rescue of *Drosophila* cephalic defects by the human *Otx* genes. *Proc. Natl. Acad. Sci. USA* **95:** 3737–3742.

Pannese, M., Polo, C., Andreazzoli, M., Vignali, R., Kablar, B., Barsacchi, G., and Boncinelli, E. (1995). The *Xenopus* homologue of *Otx2* is a maternal hoemobox gene that demarcates and specifies anterior body regions. *Development* **121:** 707–720.

Perrimon, N. (1994). The genetic basis of patterned baldness in *Drosophila*. *Cell* **76:** 781–784.

Rhinn, M., Dierich, A., Shawlot, W., Behringer, R. R., Le Meur, M., and Ang, S.-L.(1998). Sequential roles for *Otx2* in visceral endoderm and neuroectoderm for forebrain and midbrain induction and specification. *Development* **125:** 845–856.

Royet, J., and Finkelstein, R. (1995). Pattern formation in *Drosophila* head development: The role of the *orthodenticle* homeobox gene. *Development* **121:** 3561–3572.

Rubenstein, J. L. R., and Beachy, P. A. (1998). Patterning the embryonic forebrain. *Curr. Op. Neurobiol*. **8:** 18–26.

Rubenstein, J. L. R., Shimamura, K., Martinez, S., and Puelles, L. (1998). Regionalization of the prosencephalic neural plate. *Annu. Rev. Neurosci*. **21:** 445–477.

Ruiz i Altaba, A. (1993). Induction and axial patterning of the neural plate: Planar and vertical signals. *J. Neurobiology* **24:** 1276–1304.

Ruiz i Altaba, A. (1994). Pattern formation in the vertebrate neural plate. *Trends Neurosci*. **17:** 233–243.

Ruiz i Altaba, A. (1998). Deconstructing the organizers. *Nature* **391:** 748–749.

Sharman, A. C., and Brand, M. (1998). Evolution and homology of the nervous system: Cross-phylum rescues of *otd/Otx* genes. *Trends Genet*. **14:** 211–214.

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

Simeone, A., Acampora, D., Mallamaci, A., Stornaiuolo, A., D'Apice, M. R., Nigro, V., and Boncinelli, E. (1993). A vertebrate gene related to *orthodenticle* contains a homeodomain of the *bicoid* class and demarcates anterior neuroectoderm in the gastrulating mouse embryo. *EMBO J*. **12:** 2735–2747.

Simeone, A., Avantaggiato, V., Moroni, M. C., Mavilio, F., Arra, C., Cotelli, F., Nigro, V., and Acampora, D. (1995). Retinoic acid induces stage-specific antero-posterior transformation of rostral central nervous system. *Mech. Dev*. **51:** 83–98.

Suda, Y., Matsuo, I., Kuratani, S., and Aizawa, S. (1996). *Otx1* function overlaps with *Otx2* in development of mouse forebrain and midbrain. *Genes to Cells* **1:** 1031–1044.

Thomas, P., and Beddington, R. (1996). Anterior primitive endoderm may be responsible for patterning the anterior neural plate in the mouse embryo. *Curr. Biol*. **6:** 1487–1496.

Thor, S. (1995). The genetics of brain development: Conserved programs in flies and mice. *Neuron* **15:** 975–977.

Varlet, I., Collignon, J., and Robertson, E. J. (1997). *nodal* expression in the primitive endoderm is required for specification of the anterior axis during mouse gastrulation. *Development* **124:** 1033–1044.

Wray, G. A., Levinton, J. S., and Shapiro, L. H. (1996). Molecular evidence for deep precambrian divergences among metazoan phyla. *Science* **274:** 568–573.

Younossi-Hartenstein, A., Green, P., Liaw, G. J., Rudolph, K., Lengyel, J., and Hartenstein, V. (1997). Control of early neurogenesis of the *Drosophila* brain by the head gap genes *tll*, *otd*, *ems* and *btd*. *Dev. Biol*. **182:** 270–283.

Received September 1, 1998
Revised November 3, 1998
Accepted November 3, 1998