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EMBO MEMBER'S REVIEW

Otx1 and *Otx2* in the development and evolution of the mammalian brain

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In the last decade, a number of genes related to the induction, specification and regionalization of the brain were isolated and their functional properties currently are being dissected. Among these, *Otx1* and *Otx2* play a pivotal role in several processes of brain morphogenesis. Findings from several groups now confirm the importance of *Otx2* in the early specification of neuroectoderm destined to become fore–midbrain, the existence of an *Otx* gene dosage-dependent mechanism in patterning the developing brain, and the involvement of *Otx1* in corticogenesis. Some of these properties appear particularly fascinating when considered in evolutionary terms and highlight the central role of *Otx* genes in the establishment of the genetic program defining the complexity of a vertebrate brain. This review deals with the major aspects related to the roles played by *Otx1* and *Otx2* in the development and evolution of the mammalian brain.

Keywords: brain evolution/brain patterning/
corticogenesis/head organizer/visceral endoderm

Introduction

One of the most fascinating goals of molecular embryology is to understand the molecular mechanisms controlling induction, specification and regionalization of the brain. In vertebrates, a remarkable amount of data has been collected in recent years on the role of genes which are candidates for the control of developmental programs underlying brain morphogenesis. Most of these genes are the vertebrate homologs of *Drosophila* genes coding for signal molecules or transcription factors (Lemaire and Kodjabachian, 1996; Tam and Behringer, 1997; Rubenstein *et al.*, 1998). Among these, the *orthodenticle* group is defined by the *Drosophila orthodenticle* (*otd*) and the vertebrate *Otx1*, *Otx2* and *Crx* genes which contain a bicoid-like homeodomain (Finkelstein and Boncinelli, 1994; Chen *et al.*, 1997; Freud *et al.*, 1997). The *Drosophila otd* gene is expressed at the anterior pole of the blastoderm embryo and later predominantly in the developing rostralmost brain neuromere (Finkelstein and Perrimon, 1990; Finkelstein *et al.*, 1990; Cohen and Jürgens, 1990, 1991; Hirth *et al.*, 1995; Younossi-Hartenstein *et al.*, 1997).

Together with two additional genes, the homeobox-containing gene *empty spiracles* (*ems*) and the zinc-finger gene *buttonhead* (*btd*), *otd* defines an ordered and partially overlapping expression pattern including adjacent head segments. Mutations in each of these three genes cause the loss of anterior head segments where they are expressed, suggesting that they might act as gap genes operating along the cephalic segments.

In the mouse, two cognates for *otd* (*Otx1* and *Otx2*) and two for *ems* (*Emx1* and *Emx2*) were identified by virtue of the high conservation in their homeobox sequences (Simeone *et al.*, 1992a,b, 1993). Their expression patterns along the developing brain of 9.5 days post-coitum (d.p.c.) embryos showed a remarkable similarity to the *Drosophila* counterpart genes and suggested that they might be part of a general control system operating in the brain and different from that coded by the HOX complexes controlling the hindbrain and spinal cord (Holland *et al.*, 1992; Simeone *et al.*, 1992a; Krumlauf, 1994). Further evidence deriving from expression data also suggested roles for *Otx1* in corticogenesis, for *Otx2* in the early specification of rostral neuroectoderm and for both genes in sense organ development (Simeone *et al.*, 1993; Ang *et al.*, 1994; Frantz *et al.*, 1994). These potential roles have been the subject of intense study and are now being elucidated by genetic analyses.

Early Otx2 requirement for rostral CNS specification

Fate and patterning of tissues depend on the activity of organizer cells emanating signals to a responding tissue which undergoes morphogenetic changes resulting in a specific differentiated fate (Spemann and Mangold, 1924; Waddington, 1932; Gurdon, 1987). The first evidence of an organizer comes from transplantation experiments in amphibians, in which the dorsal lip of an early blastopore induces a new, ectopic secondary axis when transplanted to the ventral side of a host embryo (Spemann and Mangold, 1924). Functionally-equivalent organizing regions named Hensen's node and the node have been identified in chick and mouse embryos, respectively.

It is also known that early patterning of the central nervous system (CNS) primordium is controlled by distinct mechanisms involving vertical signals directed from axial mesendoderm to the surrounding neural plate, and planar signals acting through the neuroectodermal plane (Doniach, 1993; Ruiz i Altaba, 1993, 1994; see Figure 1). In this context, it has been shown recently that in *zebrafish*, a small group of ectodermal cells located in the prospective head region is required for the patterning and survival of the anterior brain (Houart *et al.*, 1998; Ruiz i Altaba, 1998). However, a large body of evidence indicates that the anterior region of the primitive visceral endoderm in mouse as well as the leading edge of the involuting endoderm in *Xenopus* also play a crucial role in head organizer activity (Bouwmeester *et al.*, 1996; Thomas and Beddington, 1996; Varlet *et al.*, 1997; Thomas *et al.*,

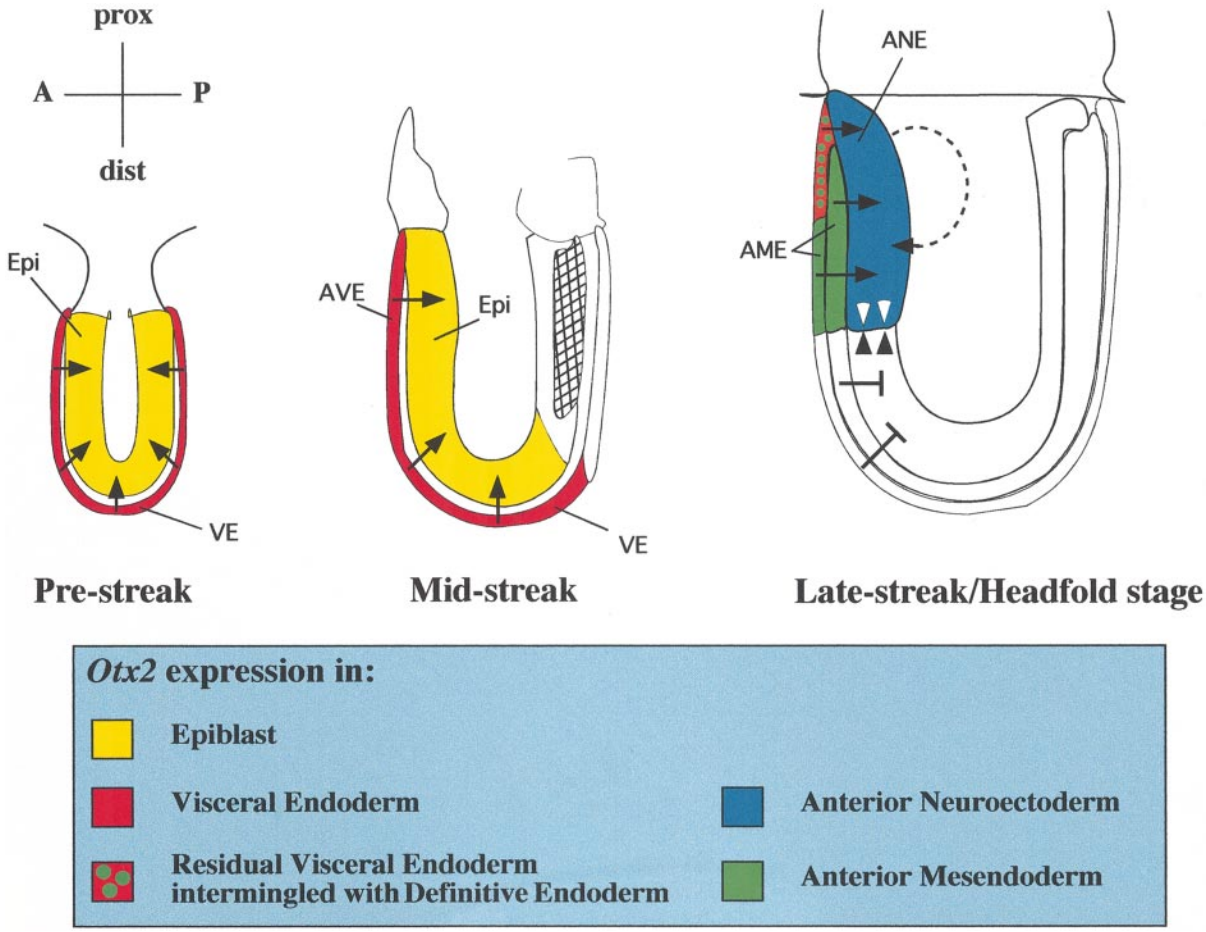

Fig. 1. *Otx2* expression and hypothetical *Otx2*-mediated tissue interactions during murine gastrulation. At the pre-streak stage, *Otx2* is transcribed in the entire visceral endoderm and epiblast. As the primitive streak progresses, *Otx2* expression gradually is restricted to the anterior third of the embryo and, at the late streak/headfold stage, includes all three germ layers. At this stage, the anterior neuroectoderm is underlined by node-derived axial mesendoderm and, in the most anterior region, by residual visceral endoderm cells intermingled with definitive endoderm cells. Tissue recombination experiments, chimeric embryos and *Otx2* null embryos led to the hypothesis that there exists an early streak *Otx2*-dependent signal(s) (arrows) emitted from the visceral endoderm, directed to the epiblast and required for early neural plate specification and primitive streak organization. At the late streak/headfold stage, a positive vertical signal (arrows) from the anterior node-derived axial mesendoderm may act to maintain *Otx2* expression in the surrounding neuroectoderm and co-exist with that coming from the residual visceral endoderm. Similarly, a negative signal (T), mimicked by retinoic acid and deriving from posterior axial mesendoderm, might contribute to defining the posterior border of *Otx2* expression together with planar interactions throughout the neuroectodermal plane (arrowheads) between different gene products (e.g. *Otx2* and *Gbx-2*). Finally, from the headfold stage onwards (0–8 somite stage), an *Otx2*-autonomous function (circular arrow) might be required for maintenance of fore–midbrain regional identities (Acampora *et al.*, 1997; Rhinn *et al.*, 1998).

1998). An increasing amount of data (see below) supports the importance of the role of *Otx2* in the specification and patterning of the anterior neural plate. These findings lead to a tentative model for *Otx2* action during gastrulation (Figure 1).

Otx2 is transcribed in the cells that are believed to emit signals in early specification and patterning of the neural plate (the anterior visceral endoderm and prechordal mesendoderm) as well as in those responding to these instructing signals (the epiblast and anterior neuroectoderm) (Simeone *et al.*, 1993; Ang *et al.*, 1994) (Figure 1).

At the onset of gastrulation, *Otx2* is required in the visceral endoderm to maintain its transcription in the epiblast and to mediate *Otx2*-dependent signals directed from the visceral endoderm to the epiblast. Embryos lacking *Otx2* fail to generate this signal in the visceral endoderm, and display an abnormal mesoderm organization and the absence of the rostral neuroectoderm (see below). From early to late streak stage, this signal persists in the visceral endoderm to maintain *Otx2* transcription in the surrounding ectoderm. It is not clear how the posterior repression of *Otx2* is mediated during mid–late streak formation, though either the gradual anterior displacement of the visceral endoderm or primitive streak progression may be involved. At the headfold stage, a positive signal from the node-derived anterior mesendoderm is required for *Otx2* transcription in the surrounding neuroectoderm and possibly contributes to the maintenance of the anterior character, while a negative signal from the posterior mesendoderm represses *Otx2*, presumably contributing to the positioning of its posterior border (Ang *et al.*, 1994; Foley *et al.*, 1997). In this context, impaired axial mesendoderm of *Otx2–/–* embryos are likely to be a consequence of the *Otx2* requirement at earlier stages in the visceral endoderm (see below). Furthermore, the *Otx2* posterior border might also result from interaction with factors (e.g. retinoic acid) and/or other genes expressed through the neuroectoderm. *Gbx2* might be a

good candidate, since at the headfold stage its anterior border of expression is adjacent to that of *Otx2* and, in *Gbx2–/–* mice, a posterior expansion of the *Otx2*-expressing territory is evident (Wassarman *et al.*, 1997). Finally, from the headfold stage onwards, *Otx2* might be required autonomously in the neuroectoderm to specify and maintain fore–midbrain identity (Acampora *et al.*, 1997; Rhinn *et al.*, 1998; A.Simeone, unpublished results).

In vivo embryological and genetic manipulation experiments have contributed to the above model. In mouse, *Otx2* null embryos die early in embryogenesis, lack the rostral neuroectoderm fated to become forebrain, midbrain and rostral hindbrain, and show major abnormalities in their body plan (Acampora *et al.*, 1995; Matsuo *et al.*, 1995; Ang *et al.*, 1996). Heterozygous *Otx2+/–* embryos in an appropriate genetic background show defects of the head such as serious brain abnormalities and craniofacial malformations, which are reminiscent of otocephalic phenotypes (Matsuo *et al.*, 1995).

The analysis of *Otx2* null embryos reveals that at the late streak stage, the rostral neuroectoderm is not identified and the primitive streak as well as the node-derived cells of the axial mesendoderm are severely impaired. Therefore, the resulting headless phenotype has been interpreted as the consequence of the abnormal development of prechordal axial mesendoderm which lacks head organizer activity. Indeed, a similar explanation has been argued for the headless phenotype of *Lim1–/–* mutants (Shawlot and Behringer, 1995). However, in embryos in which *Otx2* is replaced with a *LacZ* reporter gene, the first abnormality is already detected at the early streak stage (Acampora *et al.*, 1995). At this stage, *LacZ* staining and transcription are abolished in the epiblast while they remain high in all the visceral endoderm of *Otx2–/–* embryos. Furthermore, *goosecoid* (*gsc*) transcripts, which normally label early node precursor cells having inducing properties (Izpisùa-Belmonte *et al.*, 1993), are undetectable or confined to the proximal region of *Otx2–/–* embryos.

Thus, since *Otx2* is already transcribed from the earliest stages (unfertilized egg in *Xenopus* and at least the morula in mouse), these data indicate that the maintenance of *Otx2* transcription in the epiblast cells requires at least one normal allele expressed in the visceral endoderm, while *Otx2* transcription in the visceral endoderm is independent of the presence of a normal allele. Therefore, in contrast to the former interpretation deduced from the late gastrula phenotype, these results support the possibility that abnormal primitive streak organization and the headless phenotype might be determined very early at the pre-early streak stages by an impairment of visceral endoderm properties. These visceral endoderm properties could correspond to an *Otx2*-dependent signal(s) with the epiblast cells as the target (Figure 1). In this context, it is noteworthy that the chick hypoblast is required for the correct formation of the primitive streak (Stern, 1992) and that the chick hypoblast and the murine visceral endoderm might share similar roles in primitive streak organization. Moreover, an increasing amount of data strongly supports a role for the anterior visceral endoderm in head organizer activity: (i) removal of a patch of anterior visceral endoderm cells expressing the *Rpx/Hesx1* gene prevents the subsequent expression of the gene in the rostral headfolds which become reduced and abnormally patterned (Thomas and Beddington, 1996; Dattani *et al.*, 1998); (ii) chimeric embryos composed of wild-type epiblast and *nodal–/–* visceral endoderm are found to be heavily impaired in rostral CNS development (Varlet *et al.*, 1997); (iii) transplantation of axial mesoderm in mouse induces a secondary axis lacking the most anterior neural tissues (Beddington, 1994); (iv) in *Xenopus*, the expression of the secreted molecule coded by the *cerberus* gene is restricted to the leading edge of the involuting endoderm and represents a potent head inducer (Bouwmeester *et al.*, 1996; Bouwmeester and Leyns, 1997); and (v) most of the genes expressed in the node or in the axial mesendoderm cells at the mid–late streak stage are also expressed in the anterior visceral endoderm. Together, these findings reinforce the idea that in mouse the organizer might be split into at least two embryonic regions operating at different stages to specify head and trunk organizer signals (Thomas and Beddington, 1996; Belo *et al.*, 1997; Ruiz i Altaba, 1998).

The relevance of *Otx2* in the anterior visceral endoderm recently has been confirmed by generating murine chimeric embryos containing *Otx2–/–* epiblast cells and wild-type visceral endoderm or vice versa (Rhinn *et al.*, 1998). In these experiments, the rescue of the early neural plate by wild-type visceral endoderm suggests both an *Otx2*-mediated role of the visceral endoderm in early neural plate specification and an *Otx2* cell-autonomous requirement in the neuroepithelium. Conversely, when chimeric embryos consist of an *Otx2–/–* visceral endoderm and an *Otx2+/+* epiblast, none of the phenotypic features of *Otx2–/–* embryos are rescued (Rhinn *et al.*, 1998). This result also supports the argument, as previously suggested in *Otx2–/–* mice (Acampora *et al*., 1995), that an impaired axial mesendoderm in *Otx2–/–* embryos is a consequence of an *Otx2* requirement at earlier stages in the visceral endoderm. It is worth noting that the *Otx2*, *Lim1* and murine *cerberus* genes are all co-expressed in the anterior visceral endoderm, thus suggesting that they may overlap in the earliest genetic pathway involved in organizing the head (Belo *et al*., 1997; Tam and Behringer, 1997).

Additional data indicating that *Otx2* is responsive to inductive interactions between ectoderm and mesendoderm came from explant-recombination experiments in gastrulating mouse embryos, showing that a positive signal from the anterior mesendoderm of headfold stage embryos is able to maintain *Otx2* expression in the anterior ectoderm of early streak embryos, and that a negative signal from the posterior mesendoderm, mimicked by exogenous retinoic acid, represses *Otx2* expression in the anterior ectoderm of late streak embryos (Ang *et al.*, 1994). Similar interactions have also been demonstrated in *Xenopus* (Blitz and Cho, 1995).

The possibility that retinoic acid might contribute to distinguishing between fore–midbrain and hindbrain at an early stage by controlling *Otx2* expression is supported by the finding that the administration of exogenous retinoic acid at the mid–late streak stage represses early *Otx2* expression in both the axial mesendoderm and the posterior neural plate (Ang *et al.*, 1994; Simeone *et al.*, 1995; Avantaggiato *et al.*, 1996). This repression correlates with the appearance of microcephalic embryos showing early anteriorization of *Hoxb1* expression, hindbrain expansion (Sive and Cheng, 1991; Conlon and Rossant, 1992;

Marshall *et al.*, 1992; Krumlauf, 1994), loss of forebrain molecular and morphological landmarks and gain of midbrain molecular markers in the most anterior neuroectoderm (Simeone *et al.*, 1995; Avantaggiato *et al.*, 1996). Moreover, *Otx2* responsiveness to retinoic acid application is a common feature in different species including *Xenopus* and chick (Bally-Cuif *et al.*, 1995; Pannese *et al.*, 1995). Nevertheless, the question of whether the interaction between endogenous retinoic acid and *Otx2* is a physiological event in rostral CNS demarcation still remains unsolved.

Finally, experiments performed in *Xenopus* embryos also highlight the *Otx2* involvement in the early specification of rostral CNS and, to some extent, complement the results obtained in mouse. In fact, microinjection of synthetic *Otx2* RNA results in an abnormal reduction in the size of tail and trunk structures, and in the appearance of a second cement gland (Blitz and Cho, 1995; Pannese *et al.*, 1995). These phenotypes have been interpreted either as a possible *Otx2*-mediated interference with movements of extension and convergence during gastrulation (for trunk and tail reduction) or as an *Otx2* requirement in the specification of most anterior head structures (for the ectopic cement gland). Moreover, by using a dexamethasone-inducible OTX2 protein, it has been shown that the *Xenopus Otx2* activity is regulated by a regionally restricted factor(s), and that the cement gland-specific gene *Xcg* is a direct target of the *Otx2* gene product (Gammill and Sive, 1997).

Otx1 is required for corticogenesis and sense organ development

The cerebral cortex is one of the most complex and fascinating areas of the brain. Neurons within the neocortex are organized in a highly ordered and differentiated array and arise from dividing progenitors of a simple neuroepithelium in which cells appear morphologically indistinguishable (McConnell, 1995).

During murine embryogenesis, *Otx1* expression is detected first at the 1–3 somite stage (8 d.p.c.) throughout the forebrain and midbrain neuroepithelium (Simeone *et al.*, 1993). In particular, during cerebral cortex development, *Otx1* initially is transcribed throughout the entire dorsal telencephalic neuroepithelium; subsequently, towards the stages corresponding to the generation of neurons belonging to the deep cortical layers, it is restricted to the ventricular zone and, later, at the end of gestation, it becomes prominent in the cortical plate consisting of post-migratory neurons of layers 5 and 6 (Frantz *et al.*, 1994). At the end of gestation, the *Otx1* signal is weakened in the ventricular zone and, postnatally, it is expressed prevalently in a subset of neurons in layers 5 and 6 (Frantz *et al.*, 1994).

Moreover, *Otx1* is also expressed in restricted derivatives of olfactory, visual and acoustic sense organs (Simeone *et al.*, 1993).

To gain an insight into its functional role, *Otx1* null mice have been generated in two different laboratories. Due to the different genetic background, in one case *Otx1–/–* mice die at birth (Suda *et al.*, 1996), while in the other only 30% of them die at the weaning stage (Acampora *et al.*, 1996). Both cases attest to the involvement of *Otx1* in specific brain areas and at specific developmental stages. In particular, the cortex of adult *Otx1–/–* brains is reduced, and the identification of the neuronal layers in the temporal and perirhinal areas is difficult. This suggests that *Otx1* is required for the development of the entire dorsal telencephalic cortex, with a more specific effect in the temporal and perirhinal areas, where events specifying neuronal identity also might be affected. The cortical phenotype is well correlated with a perturbation of early proliferative potentialities of neuronal precursors (Acampora *et al.*, 1998a). Abnormalities identified in sense organs indicate that *Otx1* is also required to specify the ciliary process in the eye and the lateral semicircular duct in the inner ear (Acampora *et al.*, 1996).

It is noteworthy that *Otx1* gene disruption generates a clear epileptic phenotype and occasional movement disorders including high speed turning behavior (Acampora *et al.*, 1996). Epilepsy, one of the most common conditions in human pathology affecting 0.5–2% of the population, includes a variety of disorders related to electrical activity abnormalities of the brain. It has been suggested that cortical dysgenesis might be common and responsible for the so-called cryptogenic epilepsies (Meencke and Janz, 1984; Raymond *et al.*, 1995). Although several etiological factors have been proposed to cause cortical dysgenesis, genes affecting events early in corticogenesis appear to be the major candidates (Noebels, 1996). Therefore, mutations in the *Otx1* gene might be responsible for a cortical dysgenesis disorder leading to epilepsy even in humans, although no such mutations have been identified so far.

Otx genes in brain regionalization

Events underlying the antero-posterior patterning of the CNS begin to be established during the early gastrulation stage and lead to the generation of distinct transverse domains along the antero-posterior (A/P) body axis. These early events require interactions among different tissues (the anterior visceral endoderm, axial mesendoderm and ectoderm), and several genes contribute to the achievement of these (Tam and Behringer, 1997; Rubenstein *et al.*, 1998). It has been proposed that organizing centers are generated at the boundary between juxtaposed differently specified territories where cooperative interactions result in the production of signaling molecules with inducing properties (Meinhardt, 1983).

An inductive signal may be generated either at the boundary between adjacent transverse domains or in a restricted longitudinal domain running all along the A/P axis. In both cases, target tissues activate specific differentiating programs depending on their ability to respond to the inductive signal. Territorial competence and inductive signals produced by organizing centers are the main contributors towards the establishment of the morphogenetic fate of distinct brain areas.

Elegant transplantation experiments indicate both the presence of an organizer at the isthmic constriction of the mesencephalic–metencephalic (mes–met) junction and the existence of a different territorial competence between the brain regions located rostrally (prosomeres 3–6) and posteriorly to the zona limitans intrathalamica (mesencephalon and prosomeres 1 and 2) (Martinez *et al.*, 1991;

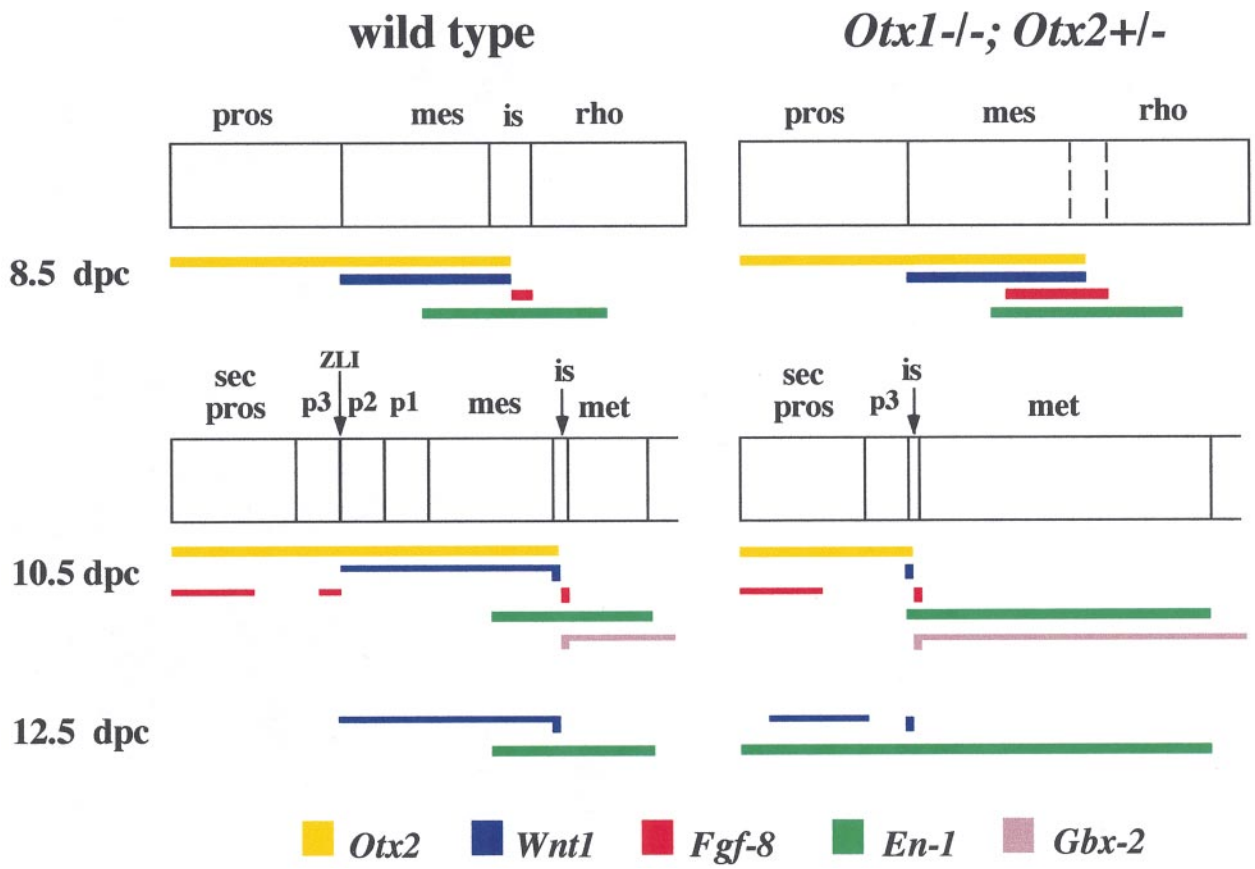

Fig. 2. *Otx* gene dosage effect on brain patterning. In wild-type embryos, at 8.5 d.p.c., the expression patterns of *Otx2*, *Wnt-1* and *En-1* define broader regions corresponding to the prosencephalon and mesencephalon (*Otx2*), mesencephalon (*Wnt-1*) and posterior mesencephalon and rostral rhomboencephalon (*En-1*), while *Fgf-8* expression identifies the isthmic primordium. During subsequent development, the brain areas acquire a more specific regional identity in parallel with a progressive restriction of molecular markers such as *Otx2*, *Wnt-1*, *Fgf-8*, *En-1* and *Gbx-2* which define the molecular code of the wild-type isthmic organizer in the mes–met region. In *Otx1–/–; Otx2+/–* mutants, at 8.5 d.p.c., early prosencephalic, mesencephalic and rostral rhomboencephalic areas are defined correctly by *Otx2*, *Wnt-1* and *En-1* expression patterns, while *Fgf-8* expression is abnormally broader and invades the presumptive mesencephalic area, thus indicating an early perturbation in the isthmic primordium (dashed line). At 10.5 d.p.c., the morphology and regional identities of *Otx1–/–; Otx2+/–* embryos show dramatic morphological perturbations, and the isthmic molecular code becomes anteriorized coordinately in the area that would correspond to the prosomere 2 while the mesencephalic territory lacks its characteristic molecular features (*Otx2* and *Wnt-1* expression) and acquires metencephalic markers (*Gbx-2*, *En-1* expression). Interestingly, at 12.5 d.p.c., the secondary prosencephalon expresses mesencephalic markers such as *En-1* and *Wnt-1* even though the expression of prosencephalic markers is retained. Abbreviations: pros, prosencephalon; mes, mesencephalon; rho, rhomboencephalon; sec pros, secondary prosencephalon; met, metencephalon; is, isthmus; ZLI, zona limitans intrathalamica; p1, p2, p3, prosomeres 1, 2 and 3.

Marin and Puelles, 1994) (Figure 2). Two relevant signal molecules coded by the fibroblast growth factor-8 gene (*Fgf-8*) (Crossley and Martin, 1995; Crossley *et al.*, 1996; Lee *et al.*, 1997; Meyers *et al.*, 1998) and *Sonic hedgehog* (*Shh*) (Echelard *et al.*, 1993; Martì *et al.*, 1995; Roelink *et al.*, 1995; Chiang *et al.*, 1996; Ericson *et al.*, 1996) are transcribed locally at the isthmic constriction and zona limitans intrathalamica, respectively. *Fgf-8* midbrain-inducing properties have been demonstrated (Crossley *et al.*, 1996) while it can be hypothesized that *Shh* has a similar role in organizing tissue and/or in conferring different regional competence between territories rostral and caudal to the zona limitans intrathalamica (Martinez *et al.*, 1991; Figdor and Stern, 1993; Rubenstein *et al.*, 1994, 1998; Bally-Cuif and Wassef, 1995; Crossley *et al.*, 1996).

It is crucial to determine the molecular mechanisms necessary to specify adjacent territories with a different identity (e.g. mesencephalon and metencephalon), and in turn to allow the correct positioning of an organizer (e.g. isthmic organizer). The mes–met junction is molecularly defined by the *Wnt-1* expression ring and the posterior borders of *Otx1* and *Otx2* on the mesencephalic side, and by the *Fgf-8* expression ring and the anterior borders of *Gbx2* and *Pax2* on the metencephalic side (Bally-Cuif and Wassef, 1995; Ang, 1996; Joyner, 1996; Millet *et al.*, 1996) (Figure 2). Findings collected from expression data (Simeone *et al.*, 1992a, 1993), *Otx2* null mice (Acampora *et al.*, 1995; Matsuo *et al.*, 1995; Ang *et al.*, 1996), transplantation experiments (Millet *et al.*, 1996) and retinoic acid-induced phenocopies (Simeone *et al.*, 1995; Avantaggiato *et al.*, 1996) support the possibility that *Otx* genes might contribute to mes–met development and, indeed, two recent reports have demonstrated their involvement in this process (Acampora *et al.*, 1997; Suda *et al.*, 1997).

In the first report (Acampora *et al.*, 1997), mice carrying only one functional copy of *Otx2* (*Otx1–/–; Otx2+/–*) show molecular and morphological transformation of the caudal diencephalon (prosomeres 1 and 2) and mesencephalon into an enlarged metencephalon, and the acquisition

of mesencephalic molecular features in the telencephalon. The observed repatterning was assessed by studying the expression pattern of genes functionally involved in the establishment of the mes–met region such as *Wnt-1*, *En-1* and *Fgf-8* (Bally-Cuif and Wassef, 1995; Joyner, 1996; Rubenstein *et al.*, 1998). While in *Otx1–/–; Otx2+/–* embryos at 8.5 d.p.c., the expression domains of *Wnt-1* and *En-1* (two early markers of the mesencephalic and metencephalic regions) are unaffected, *Fgf-8* distribution, in contrast, is broader and invades adjacent rostral territory (Figure 2). A few hours later, both *Wnt-1* and *Fgf-8* fail to form their narrow stripes at the mes–met boundary, and *Fgf-8* transcripts are detected along all the presumptive rostral brain. At 10.5 d.p.c., the repatterning process begins to be evident, with the transformation of the mesencephalon into the metencephalon, the establishment of an isthmic-like structure in the caudal diencephalon and, by 12.5 d.p.c., with the telencephalic acquisition of mesencephalic features such as the expression of *En-2* and *Wnt-1* genes (Figure 2). These findings indicate that the repatterning observed is triggered by the early *Fgf-8* misexpression in response to a critical low level of *Otx* gene products (Acampora *et al.*, 1997).

In the second report (Suda *et al.*, 1997), similar conclusions were drawn from the analysis of double heterozygous embryos (*Otx1+/–; Otx2+/–*) showing, in a different genetic background, defects similar to those reported in *Otx1–/–; Otx2+/–* embryos in a BL6/DBA2 genetic background. In this report, molecular and anatomical analyses also show a severe reduction of both the mesencephalon and posterior diencephalon with an expansion of rhombomere 1 (Suda *et al.*, 1997). These studies, therefore, indicate that a crucial threshold of *Otx* gene products (possibly influenced by genetic background) is required to confer on the mesencephalic field a sufficient level of specification to allow the correct positioning of *Fgf-8*-inducing properties at the isthmic organizer.

Furthermore, the findings that in *Otx1–/–; Otx2+/–* embryos, the dorsal telencephalon acquires mesencephalic molecular features and the zona limitans intrathalamica is absent (as revealed by anatomical inspection and loss of *Shh* expression), suggest that the ability of the telencephalon to express mesencephalic genes could be related directly to the loss of the zona limitans intrathalamica and/or *Shh*-mediated signaling (Acampora *et al.*, 1997).

otd/Otx evolutionary conservation

Striking evolutionary conservation of regulatory genes that control vertebrate development is exhibited by *HOM/HOX* complexes (Lewis, 1978; Duboule and Dollé, 1989; Krumlauf, 1994; van der Hoeven *et al.*, 1996) and *ey/Pax6* genes (Callaerts *et al.*, 1997).

otd/Otx genes are also likely to have a conserved functional role in brain morphogenesis. This assumption is argued from sequence homology, which is restricted mainly to the homeodomain, and from striking similarities in their expression patterns and mutant phenotypes in *Drosophila* and mouse. In *otd* mutants, most proterocerebral neuroblasts and some deuterocerebral neuroblasts do not form, giving rise to a dramatically reduced brain (Finkelstein and Perrimon, 1990; Cohen and Jürgens, 1991; Hirth *et al.*, 1995; Thor, 1995; Reichert and Boyan, 1997; Younossi-Hartenstein *et al.*, 1997). *otd* mutants also have pattern deletions in cephalic structures. For example, in *ocelliless*, a viable *otd* allele expression in the vertex primordium is abolished, and the ocelli (light-sensing organs) and associated sensory bristles are lost (Finkelstein *et al.*, 1990). 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 the early specification and patterning of the rostral neuroectoderm, in corticogenesis and proliferation of early telencephalic neuroblasts, as well as the development of visual and acoustic sense organs (Acampora *et al.*, 1995, 1996, 1997; Matsuo *et al.*, 1995; Ang *et al.*, 1996).

To gain insight into the possibility that a basic genetic program of cephalic development might be conserved between vertebrates and invertebrates, human *Otx* genes have been introduced and overexpressed in *Drosophila otd* mutants (Leuzinger *et al.*, 1998; Nagao *et al.*, 1998) and, conversely, the murine *Otx1* has been replaced with the *Drosophila otd* gene (Acampora *et al.*, 1998a). Human *Otx1* and *Otx2* genes complement the *otd* defects allowing the rescue of brain, ventral nerve cord and cephalic defects in *Drosophila*. Moreover, their ubiquitous overexpression in the fly is able to induce ectopic neural structures (Leuzinger *et al.*, 1998). Similarly, the *Drosophila otd* gene is able to replace the mouse *Otx1* gene and fully rescue corticogenesis impairment and epilepsy, and also partially to recover eye defects and brain patterning abnormalities detected in *Otx1–/–; Otx2+/–* embryos; in contrast, the defective lateral semicircular duct of the inner ear of *Otx1–/–* mice is never recovered (Acampora *et al.*, 1998a), suggesting that the ability to specify this structure therefore represents an *Otx1*-specific property.

Despite the functional rescue observed, several aspects are still unclear. For example, the finding that homeodomains of a specific type such as the *otd* type are highly conserved might imply that they are crucial in selecting the same target sequence(s) with a very high stringency. In this connection, rescues of *Drosophila otd* and mouse *Otx1* mutant phenotypes support the possibility that they control genetic hierarchies which share, at least in part, common functional features, and that the homeodomain-mediated ability to recognize the same target sequence(s) might have been retained in evolution. In contrast, the role of coding sequences outside the homeodomain is only poorly understood, and it is important to determine whether these regions code for new functions, are evolved versions of an old function or represent a combination of old and new functions.

The rostral architectural components of the vertebrate brain, the telencephalon, the diencephalon and the mesencephalon, are clearly recognizable in vertebrates, while their existence is less clear in lower chordates (Kuhlenbeck, 1973). *otd*-related genes have been found in all chordates (Simeone *et al.*, 1992a; Bally-Cuif *et al.*, 1995; Mercier *et al.*, 1995; Pannese *et al.*, 1995; Wada *et al.*, 1996; Williams and Holland, 1996, 1998; Ueki *et al.*, 1998), where their expression is always associated with the most anterior CNS independently of the complexity acquired by this area during evolution. We propose that the architecture of this rostral *Otx*-expressing region of the CNS might have been greatly modified on the basis

of new genetic instruction(s). A posterior displacement of the mes–met boundary as well as differential proliferative properties of the rostral neuroectoderm (forebrain and midbrain) versus the more posterior neuroectoderm (hindbrain and spinal cord) might have contributed to the vertebrate-type brain respecification. In this context, it should be kept in mind that an anterior displacement of the mes–met boundary is seen in *Otx1–/–; Otx2+/–* embryos and that *Otx1–/–* mice have reduced proliferative activity in the rostral neuroepithelium (Acampora *et al.*, 1997, 1998a). Nevertheless, the rescues of *Otx1* abnormalities by *otd* and vice versa argue in favor of an evolutionary conservation of several common *otd/Otx1* properties and support the idea that conserved genetic functions required in mammalian brain development evolved in a primitive ancestor of flies and mice >500 million years ago (Wray *et al.*, 1996).

Conclusions and future directions

Published findings now indicate that *Otx1* plays important roles in patterning and terminal differentiating events of brain morphogenesis and that *Otx2* is required in early specification and patterning of the rostral CNS. Although additional aspects related to sense organ development and pituitary control of hormone production (Acampora *et al.*, 1998b) have been reported, the involvement of *Otx1* and *Otx2* in the development and evolution of the brain seems to be the most exciting aspect. Future experiments should identify *Otx1* and *Otx2* regulatory controls as well as the functional domains of their gene products and, thereby, define properties that are required to control developmental pathways or that have been created and selected during evolution to specify the greater complexity of the mammalian brain. These studies, together with the identification of a molecular partner(s) and downstream target(s), will significantly contribute to our knowledge on the morphogenesis and evolution of the mammalian brain.

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References

Acampora,D., Mazan,S., Lallemand,Y., Avantaggiato,V., Maury,M., Simeone,A. and Brûlet,P. (1995) Forebrain and midbrain regions are deleted *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. *et al.* (1998b) 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. (1996) The brain organization. *Nature*, **380**, 25–27.

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

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.

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

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. and Leyns,L. (1997) Vertebrate head induction by anterior primitive endoderm. *BioEssays*, **19**, 855–863.

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.

Callaerts,P., Halder,G. and Gehring,W.J. (1997) *Pax-6* in development and evolution. *Annu. Rev. Neurosci.*, **20**, 483–532.

Chen,S., Wang,Q.-L., Nie,Z., Sun,H., Lennon,G., Copeland,N.G., Gilbert,D.J., Jenkins,N.A. and Zack,D.J. (1997) Crx, a novel Otx-like paired-homeodomain protein, binds to and transactivates photoreceptor cell-specific genes. *Neuron*, **19**, 1017–1030.

Chiang,C., Litingtung,Y., Lee,E., Young,K.E., Cordern,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.

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

Cohen,S.M. and Jürgens,G. (1991) *Drosophila* headlines. *Trends Genet.*, **7**, 267–272.

Conlon,R.A. and Rossant,J. (1992) Exogenous retinoic acid rapidly induces anterior ectopic expression of murine *Hox-2* genes *in vivo*. *Development*, **116**, 357–368.

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.

Crossley,P.H., Martinez,S. and Martin,G.R. (1996) Midbrain development induced by FGF8 in the chick embryo. *Nature*, **380**, 66–68.

Dattani,M.T. *et al.* (1998) Mutations in the homeobox gene *HESX1/Hesx1* associated with septo-optic dysplasia in human and mouse. *Nature 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.

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

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.

Ericson,J., Morton,S., Kawakami,A., Roeling,H. and Jessell,T.M. (1996) Two crucial periods of Sonic Hedgehog signaling required for the specification of motor neuron identity. *Cell*, **87**, 661–673.

Figdor,M.C. and Stern,C.D. (1993) Segmental organization of embryonic diencephalon. *Nature*, **363**, 630–634.

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

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 homeodomain protein involved in the development the *Drosophila* nervous system and ocellar visual structures. *Genes Dev.*, **4**, 1516–1527.

Foley,A.C., Storey,K.G. and Stern,C.D. (1997) The prechordal region lacks neural inducing ability, but can confer anterior character to more posterior neuroepithelium. *Development*, **124**, 2983–2996.

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.

Freud,C.L. *et al*. (1997) Cone–rod dystrophy due to mutations in a novel photoreceptor-specific homeobox gene (*CRX*) essential for maintenance of the photoreceptor. *Cell*, **91**, 543–553.

Gammill,L.S. and Sive,H. (1997) Identification of *otx2* target genes and restrictions in ectodermal competence during *Xenopus* cement gland formation. *Development*, **124**, 471–481.

Gurdon,J.B. **(**1987) Embryonic induction—molecular prospects. *Development*, **99**, 285–306.

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.

Holland,P., Ingham,P. and Krauss,S. (1992) Mice and flies head to head. *Nature*, **358**, 627–628.

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.

Izpisùa-Belmonte,J.C., De Robertis,E.M., Storey,K.G. and Stern,C.D. (1993) The homeobox gene *goosecoid* and the origin of the organizer cells in the early chick blastoderm. *Cell*, **74**, 645–659.

Joyner,A.L. (1996) *Engrailed*, *Wnt* and *Pax* genes regulate midbrain–hindbrain development. *Trends Genet.*, **12**, 15–20.

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

Kuhlenbeck,H. (1973) *The Central Nervous System of Vertebrates*. S.Karger, Basel, Switzerland.

Lee,S.M.K., Danielian,P.S., Fritzsch,B. and McMahon,A.P. (1997) Evidence that FGF8 signalling from the midbrain–hindbrain junction regulates growth and polarity in the developing midbrain. *Development*, **124**, 959–969.

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.

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

Marin,F. and Puelles,L. (1994) Patterning of the embryonic avian midbrain after experimental inversions: a polarizing activity from the isthmus. *Dev. Biol.*, **163**, 19–37.

Marshall,H., Nonchev,S., Sham,M.H., Muchamore,I., Lumsden,A. and Krumlauf,R. (1992) Retinoic acid alters hindbrain Hox code and induces transformation of rhombomeres 2/3 into 4/5 identity. *Nature*, **360**, 737–741.

Martì,E., Bumcrot,D.A., Takada,R. and McMahon,A.P. (1995) Requirement of 19K form of Sonic hedgehog for induction of distinct ventral cell types in CNS explants. *Nature*, **375**, 322–325.

Martinez,S., Wassef,M. and Alvarado-Mallart,R.-M. (1991) Induction of a mesencephalic phenotype in the 2 day-old chick prosencephalon is preceded by the early expression of the homeobox gene *en*. *Neuron*, **6**, 971–981.

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.

McConnell,S.K. (1995) Constructing the cerebral cortex: neurogenesis and fate determination. *Neuron*, **15**, 761–768.

Meencke,H.J. and Janz,D. (1984) Neuropathological findings in primary generalized epilepsy: a study of eight cases. *Epilepsia*, **25**, 8–21.

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.

Meyers,E.N., Lewandoski,M. and Martin,G.R. (1998) An *Fgf8* mutant allelic series generated by Cre- and Flp-mediated recombination. *Nature Genet.*, **18**, 136–142.

Millet,S., Bloch-Gallego,E., Simeone,A. and Alvarado-Mallart,R.-M. (1996) Is the caudal limit of *Otx2* gene expression 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.

Noebels,J.L. (1996) Targeting epilepsy genes. *Neuron*, **16**, 241–244.

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.

Raymond,A.A., Fish,D.R., Sisodiya,S.M., Alsanjari,N., Stevens,J.M. and Shorvon,S.D. (1995) Abnormalities of gyration, heterotopias, tuberons sclerosis, focal cortical dysplasia, microdysgenesis, dysembryoplastic neuroepithelial tumor and dysgenesis of the archicortex in epilepsy. Clinical, EEG and neuroimaging features in 100 adult patients. *Brain*, **118**, 629–660.

Reichert,H. and Boyan,G. (1997) Building a brain: developmental insights in insects. *Trends Neurosci.*, **20**, 258–264.

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.

Roelink,H., Porter,J.A., Chiang,C., Tanabe,Y., Chang,D.T., Beachy,P.A. and Jessell,T.M. (1995) Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of sonic hedgehog autoproteolysis. *Cell*, **81**, 445–455.

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., Martinez,S., Shimamura,K. and Puelles,L. (1994) The embryonic vertebrate forebrain: the prosomeric model. *Science*, **266**, 578–580.

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. Neurobiol.*, **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.

Shawlot,W. and Behringer,R.R. (1995) Requirement for *Lim-1* in head organizer function. *Nature*, **374**, 425–430.

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

Simeone,A., Gulisano,M., Acampora,D., Stornaiuolo,A., D'Apice,M.R. and Boncinelli,E. (1992b) Two vertebrate homeobox genes related to the *Drosophila empty spiracles* gene are expressed in the embryonic cerebral cortex. *EMBO J.*, **11**, 2541–2550.

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.

Sive,H. and Cheng,P. (1991) Retinoic acid perturbs the expression of *Xhox.lab* genes and alters mesodermal determination in *Xenopus laevis*. *Genes Dev.*, **5**, 1321–1332.

Spemann,H. and Mangold,H. (1924) Über induktion von Embryonanlagen durch Implantation artfremder Organisatoren. *Wilhelm Roux's Arch. Entw. Mech. Organ.*, **100**, 599–638.

Stern,C.D. (1992) Mesoderm induction and development of the embryonic axis in amniotes. *Trends Genet.*, **8**, 158–163.

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

Suda,Y., Matsuo,I. and Aizawa,S. (1997) Cooperation between *Otx1* and *Otx2* genes in developmental patterning of rostral brain. *Mech. Dev.*, **69**, 125–141.

Tam,P.P.L. and Behringer,R.R. (1997) Mouse gastrulation: the formation of a mammalian body plan. *Mech. Dev.*, **68**, 3–25.

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.

Thomas,P.Q., Brown,A. and Beddington,R.S. (1998) *Hex*: a homeobox gene revealing peri-implantation asymmetry in the mouse embryo and an early transient marker of endothelial cell precursors. *Development*, **125**, 85–94.

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

Ueki,T., Kuratani,S., Hirano,S. and Aizawa,S. (1998) *Otx* cognates in a lamprey, *Lampetra japonica. Dev. Genet. Evol.*, **208**, 223–228.

van der Hoeven,F., Zakany,J. and Duboule,D. (1996) Gene transpositions in the HoxD complex reveal a hierarchy of regulatory controls. *Cell*, **85**, 1025–1035.

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.

Wada,S., Katsuyama,Y., Sato,Y., Itoh,C. and Saiga,H. (1996) *Hroth* an orthodenticle-related homeobox gene of the ascidian, *Halocynthia roretzi*: its expression and putative roles in the axis formation during embryogenesis. *Mech. Dev.*, **60**, 59–71.

Waddington,C.H. (1932) Experiments on the development of chick and duck embryos, cultivated *in vitro*. *Philos. Trans. R. Soc. Lond. B*, **221**, 179–230.

Wassarman,K.M., Lewandoski,M., Campbell,K., Joyner,A.L., Rubenstein,J.L.R., Martinez,S. and Martin,G.R. (1997) Specification of the anterior hindbrain and establishment of a normal mid/hindbrain organizer is dependent on *Gbx2* gene function. *Development*, **124**, 2923–2934.

Williams,N.A. and Holland,P.W.H. (1996) Old head on young shoulders. *Nature*, **383**, 490.

Williams,N.A. and Holland,P.W.H. (1998) Gene and domain duplication in the chordate *Otx* gene family: insights from Amphioxus Otx. *Mol. Biol. Evol.*, **15**, 600–607.

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.

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