Int. J. Dev. Biol. 46: 81-87 (2002)

Conserved genetic mechanisms for embryonic brain patterning

HEINRICH REICHERT*

Institute of Zoology, Biocenter/Pharmacenter, University of Basel, Switzerland

ABSTRACT A wealth of comparative embryological studies on the expression and function of homeotic genes and cephalic gap genes indicates that both gene groups are important for establishing and specifying the anteroposterior body axis during embryogenesis in bilaterian animals. Recently, studies of this kind have been extended to embryonic brain development in two genetic model systems, *Drosophila* and mouse. These studies demonstrate striking similarities in the pattern of expression and mode of action of these developmental control genes during embryonic patterning of the brain in both species. Thus, in both insect and mammalian species, members of the homeotic gene complex are involved in patterning the posterior brain anlage, where they control regionalized neuronal identity, and members of the cephalic gap genes, notably the *otd/Otx* gene family, are involved in patterning the anterior brain anlage where they control regionalized neurogenesis and neuronal identity. Furthermore, striking cross-phylum rescue experiments show that insect and mammalian members of the *orthodenticle* gene family can functionally replace each other in embryonic brain and CNS patterning. Comparable cross-phylum rescue experiments have now also been carried out for the *empty spiracles* cephalic gap gene family. Taken together, these experiments suggest that the genetic mechanisms involved in embryonic brain development are conserved and indicative of a common evolutionary origin of the insect and vertebrate brain. For a more extensive and quantitative investigation of the molecular conservation of developmental mechanisms for brain patterning, functional genomic experiments are now underway in *Drosophila*. These experiments exploit the advent of sequenced genome information and the technology for large scale transcript imaging, with the goal of identifying the entire set of downstream genes which is under the control of these regulatory genes in embryonic brain development.

KEY WORDS: *Drosophila, mouse, brain development, pattern formation, homeobox gene, genomics*

Introduction

Classical descriptive analyses of nervous system development in bilaterians has led to the subdivision of these animals into two large groups, the gastroneuralia and the notoneuralia. The gastroneuralia, which include arthropods, annelids and molluscs, are characterized morphologically by a ventral nerve cord consisting of metameric ganglionic structures that derive from the ventral neurectoderm. The notoneuralia, which include all chordates, are characterized by a dorsal nerve cord which derives primarily from a neural tube that invaginates from the dorsal neurectoderm. Based on differences in embryonic topography and morphogenesis of the nervous system, an independent evolutionary origin has been proposed for the nervous systems of the two bilaterian groups (gastroneuralia-notoneuralia concept; e.g. Siewing 1985; Brusca and Brusca, 1990, Nielsen, 1995). Contrasting with this notion, is the large amount of molecular genetic data that has accumulated in the last two decades which suggests that the nervous systems of gastroneuralia and notoneuralia are evolutionarily related.

Recently, neurogenetic analyses carried out in several vertebrate and invertebrate model systems have revealed striking similarities in the expression and action of regulatory gene which control neuronal embryogenesis (for reviews see Thor, 1995; Sharman and Brand, 1998; Arendt and Nübler-Jung, 1999; Reichert and Simeone, 1999). Homologous regulatory genes have been identified which control polarity, regionalization, proliferation, identity, process outgrowth, and patterning of the embryonic nervous system in a comparable manner in insects and vertebrates. Remarkable examples of evolutionary conservation in the genetic

Abbreviations used in this paper: CNS, central nervous system; *Dfd, Deformed; ems, empty spiracles; Emx1, Emx2,* mammalian homologs of *empty spiracles; Hoxa1, Hoxb1,* mammalian homologs of *labial; lab, labial; otd, orthodenticle; Otx1, Otx2,* mammalian homologs of *orthodenticle.*

***Address correspondence to:** Dr. Heinrich Reichert. Institute of Zoology, Biocenter/Pharmacenter, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland. Tel. +41-6-1267-1612. Fax +41-6-1267-1613. e-mail: Heinrich.Reichert@unibas.ch

0214-6282/2002/$25.00

Printed in Spain
www.ijdb.ehu.es

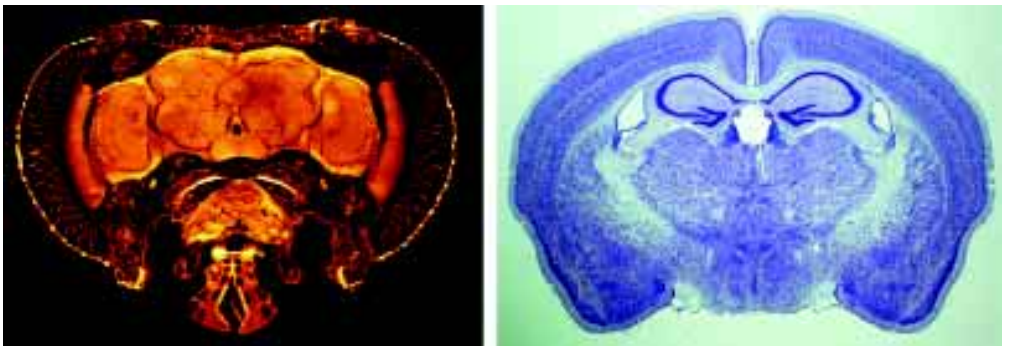

Fig. 1. Adult brains of *Drosophila* and mouse. *Coronal sections. The* Drosophila *brain is shown within the head and associated with the compound eyes. The section of the mouse brain is stained with cresyl violet and is at –1.5 bregman.*

control of CNS development include the proneural genes (Lee, 1997), the neurogenic genes (Chan and Jan, 1999), the homeotic genes (Lumsden and Krumlauf, 1996), the cephalic gap genes (Hirth and Reichert; 1999, Reichert and Simeone, 1999), the *ey/Pax6* genes (Callaerts *et al.,* 1997), and the *en/En* genes (Hanks *et al.,* 1998).

Thus, we are currently faced with an apparent paradox. Despite the obvious differences in overt neuroanatomy that characterize the nervous systems of vertebrates and invertebrates, many of the central genetic mechanisms for the control of neuronal development are remarkably similar. Evidence for the evolutionary conservation of these mechanisms becomes apparent if one leaves the superficial level of overt neuromorphology and considers the deeper molecular level of regulatory gene expression which underlies the embryogenesis of the nervous system. This is especially clear for the control of embryonic development of the brain and has been most intensively investigated using genetic *in vivo* approaches in two models systems, *Drosophila* and mouse.

Drosophila and Mouse: Two Model Systems for Studies of Brain Development and Evolution

The adult brains of *Drosophila* and mouse are markedly different both in overall size and in terms of neuroanatomical fine structure (Fig. 1). The *Drosophila* brain is composed of an anterior supraesophageal ganglion and a posterior subesophageal ganglion; the supraesophageal ganglion is subdivided into the protocerebrum (b1), deutocerebrum (b2) and tritocerebrum (b3), and the subesophageal ganglion is subdivided into the mandibular (s1), maxillary (s2) and labial (s3) neuromeres (Therianos *et al.,* 1995; Younossi-Hartenstein et. al. 1996; Reichert and Boyan, 1997). The mouse brain is divided into a rostral region that comprises the telencephalon and diencephalon (prosencephalon) as well as the mesencephalon, and into a caudal hindbrain region which has a metameric organization based on rhombomeres (Lumsden and Krumlauf, 1996).

During embryogenesis in *Drosophila*, the anterior brain anlage derives from the procephalic neurogenic region which is specified to become neuroectoderm through genetic interactions during gastrulation (Jürgens and Hartenstein, 1993). The posterior brain anlage derives from the rostral-most ventral neurogenic region and is specified in a manner similar to that of the ventral nerve cord (Doe and Skeath, 1996). During embryogenesis in the mouse, inductive interactions between germ layers during gastrulation cause an early segment-like regionalization of the developing neural tube. In the developing hindbrain, regional diversity is achieved through a process of segmentation that bears a superficial resemblance to segmentation of *Drosophila* in that seven to eight rhombomeres form by internal subdivision and have a pair-wise organization with compartment-like properties (Lumsden and Krumlauf, 1996). The segmental organization of the embryonic prosencephalon is still debated, however a number of studies suggest that this region, like the hindbrain, is subdivided into neuromeres known as prosomeres (Rubenstein *et al.,* 1994; Rubenstein *et al.,* 1998).

Recent experimental genetic evidence from *Drosophila* and mouse indicates that the expression and the function of two well known developmental control gene groups are highly conserved in embryonic brain patterning along the anteroposterior neuraxis. These gene groups are the homeotic genes and the cephalic gap genes.

Conserved Topology of Gene Expression of Homeotic *(Hox)* and *otd/Otx* Gene Families in *Drosophila* and Mouse

The homeotic genes encode homeodomain transcription factors that were first identified in *Drosophila* and subsequently found in all other bilaterian animals (Lewis, 1978; McGinnis and Krumlauf, 1992). In *Drosophila* they are arranged along the chromosome in two gene clusters known as the *Antennapedia* and the *Bithorax* complexes. The eight homeotic genes are expressed in the developing *Drosophila* embryo in spatially colinear fashion such that a 3' gene in the gene cluster is expressed more anteriorly in the embryo than its 5' neighbor (Duboule and Morata, 1994). Homeotic genes are expressed in the posterior regions of the developing brain and in the ventral nerve cord of *Drosophila* in a spatially colinear manner (Kaufman *et al.,* 1990; Hirth *et al.,* 1998).

Investigations based on molecular homology have identified *Hox* gene complexes that are homologous to the homeotic genes of *Drosophila* in all vertebrate species studied (McGinnis and Krumlauf, 1992; Lumsden and Krumlauf, 1996; Gellon and McGinnis, 1998). In many cases, the arrangement of the *Hox* genes in their chromosomal complexes correlates with their expression pattern along the anteroposterior body axis, so that spatial colinearity, in general, also applies (Duboule and Morata, 1994; Ruddle *et al.,* 1994; Capecchi, 1997; Vielle-Grosjean *et al.,* 1997). In the mouse, this is especially prominent in the developing hindbrain and spinal cord, where *Hox* gene expression patterns form an anteroposteriorly ordered set of domains along the neuraxis. The relative anteroposterior order of *Hox* gene expression in the developing mouse CNS is remarkably similar to anteroposterior order of homeotic gene expression in the *Drosophila* CNS (Fig. 2).

The cephalic gap gene *orthodenticle (otd)* encodes a homeodomain transcription factor that is required for head development and segmental patterning in *Drosophila*. The first *otd* transcripts appear at early blastoderm stages covering a broad circumferential stripe in the anterior region of the embryo that includes the anlagen of several cephalic segments as judged from blastoderm fate maps (Finkelstein *et al.,* 1990; Finkelstein and Perrimon, 1990; Cohen and Jürgens, 1990). Later in embryogenesis, expression of *otd* becomes progressively restricted to the procephalic region and to a second expression domain at the ventral midline. During neuroectoderm formation, procephalic *otd* expression covers most of the protocerebral and an adjacent part of the deutocerebral brain anlagen, and during subsequent brain regionalization neuronal *otd* expression occurs throughout most of the protocerebrum (b1) and adjacent deutocerebrum (b2) (Hirth *et al.,* 1995; Younossi-Hartenstein *et al.,* 1997).

Based on homology between homeobox sequences, homologs of the *Drosophila otd* gene have been isolated in various vertebrates including mouse (Simeone, 1998). In the mouse, the two vertebrate homologs, *Otx1* and *Otx2,* are expressed in the developing head and brain in nested and overlapping domains (Simeone *et al.,* 1992a; Simeone *et al.,* 1993; Millet *et al.,* 1996). In the early embryonic brain, *Otx2* is expressed in a broad domain that spans the forebrain and midbrain regions and has its posterior expression limit at the midbrain-hindbrain boundary. *Otx1* expression is nested within this *Otx2* expression domain anteriorly while sharing the posterior expression boundary. Thus, during embryonic patterning in the mouse and in *Drosophila*, expression of the *otd/Otx* genes extends throughout most of the anterior brain regions (Fig. 2).

Regionalization of the Brain in *Drosophila* and Mouse: Functional Conservation of Homeotic *(Hox)* and *otd/Otx* Gene Families

The function of developmental control genes in embryonic brain patterning can be most directly studied through loss-of-function experiments. In *Drosophila* loss-of-function of two homeotic genes, *labial* and *Deformed*, results in severe axonal patterning defects in the brain (Hirth *et al.,* 1998). These axonal projection defects, which include loss of commissural and longitudinal pathways, are not due to deletions in the affected neuromere since the neural progenitor cells and their postmitotic progeny are present in the mutant domain. However, the generated postmitotic cells do not extend axons or dendrites and are not contacted by axons from other parts of the brain. Moreover, these cells do not express any of the numerous neuronal molecular markers that positionally equivalent neuronal cells express in the wild type, indicating that the mutant cells in the brain do not acquire a neuronal identity. Thus, the expression of the homeotic genes *labial* and *Deformed* appears to be necessary for proper neuronal differentiation and correct establishment of regionalized neuronal identity in the posterior *Drosophila* brain.

Loss-of-function experiments have also been used to study *Hox* gene function in the mouse, notably for the *labial* homologs *Hoxa1* and *Hoxb1* (Studer *et al.,* 1998; Gavalas *et al.,* 1998). *Hoxa1* and *Hoxb1* are activated in the early neural ectoderm and by headfold stage their expression patterns have reached a sharp anterior boundary coinciding with the anterior rhombomere 4 (r4) border. In *Hoxa1-/-; Hoxb1-/-* double loss-of-function mutants, a region corresponding to r4 is formed, but r4-specific markers fail to be activated indicating the presence of a territory between r3 and r5 with an unknown identity. *Hoxa1-/-; Hoxb1-/-* double mutants also have a reduced number of facial motor neurons which appear to exit randomly from the neural tube without fasciculating. These results suggest that *Hoxa1* and *Hoxb1* act together in the specification of r4 neuronal identity and in the patterning of nerves during vertebrate hindbrain development. This mode of action is remarkably similar to that of the *Hoxa1/Hoxb1* homolog *labial* in specifying segmental neuronal identity during embryonic brain development of *Drosophila* (Hirth *et al.,* 1998).

Loss-of-function experiments demonstrate the critical involvement of *otd* in patterning the rostral embryonic brain of *Drosophila* (Hirth *et al.,* 1995; Younossi-Hartenstein *et al.,* 1997). Homozygous null mutation of *otd* results in the deletion of the protocerebral anlage due to defective neuroectoderm specification in this region. Most protocerebral neuroblasts and some deutocerebral neuroblasts are absent in the mutant, and in consequence a dramatically reduced embryonic brain is formed. This regionalized absence of brain neuroblasts in *otd* mutants correlates with the loss or reduction in expression of the *lethal of scute* gene in the *otd* mutant domain. The proneural gene *lethal of scute* is thought to be required for

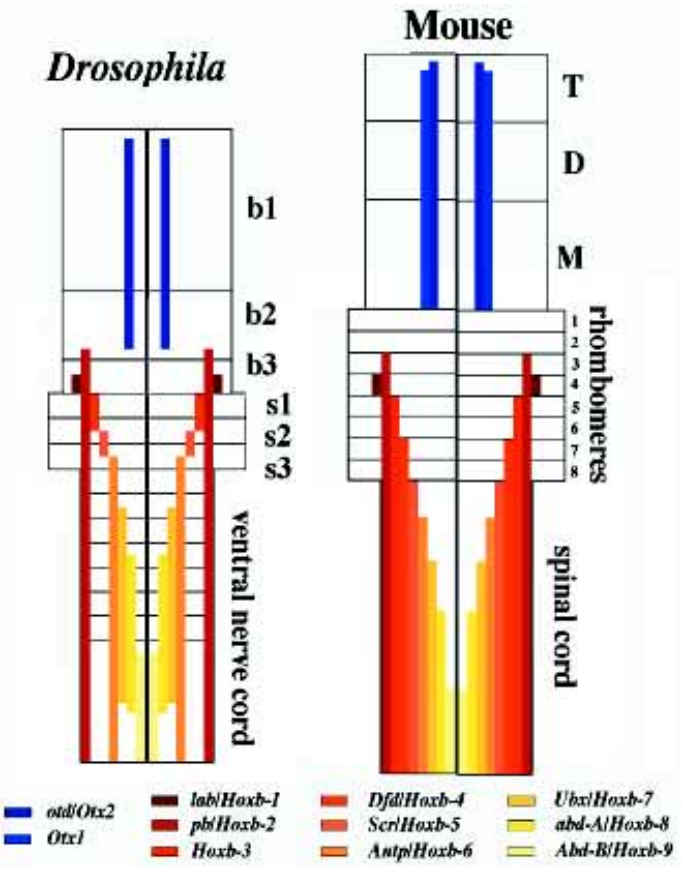

Fig. 2. Conserved anteroposterior order of gene expression in embryonic brain development. *Schematic diagram of homeotic* (Hox) *and* otd/Otx *gene expression patterns in the developing CNS of* **(left)** Drosophila *and* **(right)** *mouse. Expression domains are color coded. Anterior is towards the top. For Drosophila, gene expression corresponds to a stage 14 embryo; for the mouse, gene expression corresponds to a stage 9.5-12.5 embryo. (Modified after Reichert and Simeone, 1999).*

neurectodermal cells to acquire the competence to form neuroblasts (Younossi-Hartenstein *et al.,* 1997). In addition to defects in the anterior brain, loss of function of *otd* also causes midline defects in the ventral nerve cord resulting in deranged connectives and fused commissural axon tracts (Finkelstein *et al.,* 1990; Schmidt-Ott *et al.,* 1994; Younossi-Hartenstein *et al.,* 1997).

Loss-of-function analyses for the murine *Otx* genes show that these genes are critically required at different stages of embryonic brain development (Acampora *et al.,* 1995; Acampora *et al.,* 1996; Matsuo *et al.,* 1995; Ang *et al.,* 1996). *Otx1* null mice have spontaneous epileptic seizures and abnormalities affecting the telencephalic dorsal cortex and the mesencephalon as well as parts of the cerebellum and certain components of the acoustic and visual sense organs. In contrast, *Otx2* null mice are early embryonic lethal and lack the rostral neuroectoderm fated to become the forebrain, midbrain, and rostral hindbrain due to an impairment in early specification of the anterior neuroectoderm by the visceral endoderm. Taken together, these results indicate that the *otd/Otx* gene families have a number of essential roles in patterning the anterior embryonic brain.

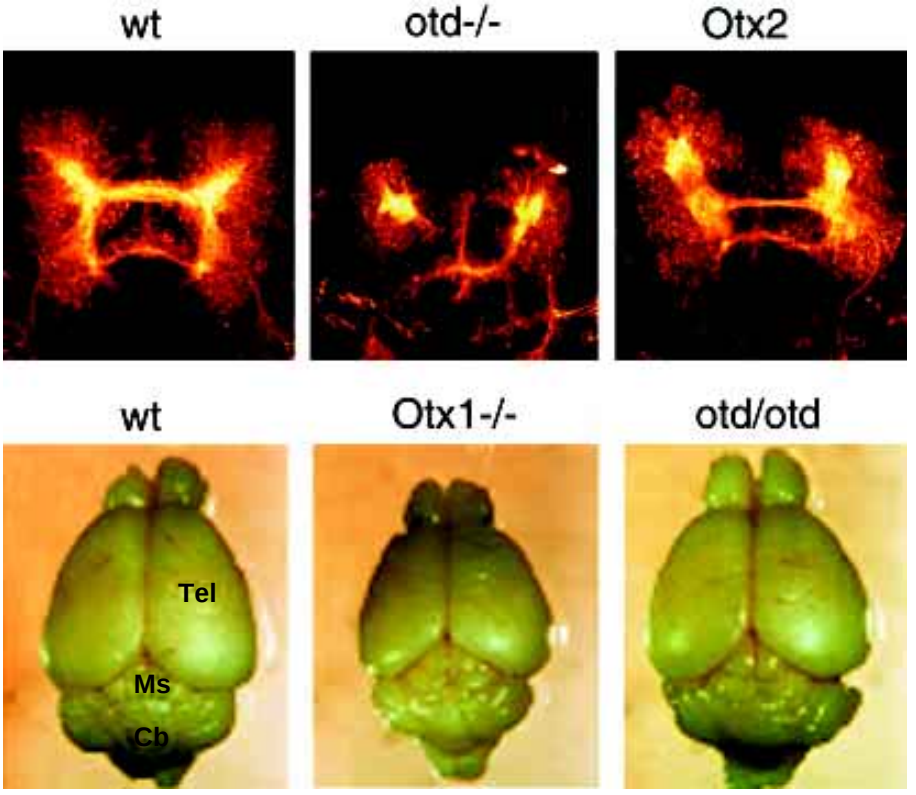

Fig. 3. *otd/Otx* cross-phylum rescue experiments in *Drosophila* and mouse. *In genetic rescue experiments, human* Otx1 *and* Otx2 *genes were overexpressed in* Drosophila otd *mutants and conversely, the murine* Otx1 *coding sequence was replaced with the* Drosophila otd *gene.* **(Upper panel)** *In Drosophila, the embryonic wildtype brain (wt) shows prominent anterior lobes interconnected by an anterior brain commissure; these structures are lost in the otd null mutant (otd-/-), but are restored by overexpression of the human* Otx2 *gene in the* otd *null mutant (Otx2).* **(Lower panel)** *In the mouse, the normal size of the wildtype adult brain (wt) is markedly reduced in the Otx1 null mutant (Otx1-/-) but is largely restored by gene replacement (knock-in) with the* Drosophila otd *gene (otd/otd). Tel, telencephalon; Ms, mesencephalon; Cb, cerebellum (Modified after Leuzinger et al., 1998 and Acampora et al., 1998).*

Cross-Phylum Rescue: Direct Evidence for Evolutionary Conservation of Developmental Control Gene Action in *Drosophila* and Mouse

Taken together, investigations of early morphogenesis and patterning in the embryonic brains of *Drosophila* and mouse reveal developmental mechanisms that are strikingly similar, and suggest an evolutionary conservation of *Hox* and *otd/Otx* genes in embryonic brain development that extends beyond gene structure to patterned expression and function. In addition to the extensive similarities in expression patterns and mutant phenotypes, *in vivo* genetic rescue experiments carried out for the *otd/Otx* gene family provide remarkable and very direct evidence for the evolutionary conservation of functional properties of these control genes in patterning of the rostral brain (Fig. 3). In these cross-phylum replacement experiments, human *Otx1* and *Otx2* genes were overexpressed in *Drosophila otd* mutants (Leuzinger *et al.,* 1998; Nagao *et al.,* 1998) and conversely, the murine *Otx1* coding sequence was replaced with the *Drosophila otd* gene (Acampora *et al.,* 1998).

In *Drosophila*, both human *Otx* genes, like the endogenous fly *otd* gene, are able to rescue the brain defects as well as the midline defects observed in *otd* null mutants. In addition, ubiquitous overexpression in wildtype embryos results in ectopic neural structures, regardless whether human *Otx* or fly *otd* are overexpressed (Leuzinger *et al.,* 1998). Similarly, the *Drosophila otd* gene is able to fully rescue corticogenesis impairment and epilepsy, and also to partially restore eye defects and brain patterning abnormalities seen in *Otx1-/-* embryos. (Acampora *et al.,* 1998). In contrast, the defective lateral semicircular duct of the inner ear of *Otx1-/-* mice is never rescued by the *Drosophila otd* gene, thus, suggesting that the ability to correctly direct the development of this structure is an *Otx1*-specific property. *Drosophila otd* is also able to partially replace *Otx1* in its cooperative interactions with *Otx2* for correct brain patterning.

Drosophila and vertebrate *otd/Otx* gene products share structural homology which is confined mainly to the homeodomain; the 60 amino acid residues of the fly OTD homeodomain differ from the homeodomains of the human OTX1 and OTX2 protein in only three and two amino acids, respectively. This implies that the extensive functional equivalence of the *otd/Otx* genes may be due to conserved developmental genetic circuits with common functional features that are controlled by the homeodomain. Thus, the *otd/Otx* gene family might be part of a general developmental genetic control system that operates in vertebrate and invertebrate brains to specify segmental identities in anterior brain and head regions. In this sense it would complement the developmental genetic control system encoded by the homeotic genes that control posterior brain and CNS regions in trunk and tail structures.

Although cross-phylum gene replacement experiments cannot formally rule out the possibility that the functional equivalence of

otd and *Otx* might have been independently acquired through convergent evolution, they argue quite strongly for an evolutionary conservation of gene function. This, in turn, suggests that common genetic mechanisms for brain development evolved in a primitive common ancestor of flies and mice and were then conserved throughout brain evolution. If this is the case, one might expect that cross-phylum rescue experiments can be carried out with other developmental control genes involved in patterning the embryonic brain. Recent experiments carried out on the *empty spiracles (ems/Emx)* cephalic gap gene family confirm this expectation.

The *Drosophila ems* gene encodes a homeodomain containing transcription factor (Dalton *et al.,* 1989; Walldorf and Gehring, 1992) and is expressed at the early cellular blastoderm stage in a single circumferential stripe at the anterior end of the embryo. Later in embryogenesis, the *ems* gene is expressed in the developing cephalic region; during neuroblast formation, *ems* is expressed in the anlage of the deutocerebrum and in the anlage of the tritocerebrum (Hirth *et al.,* 1995). In addition to its cephalic expression, *ems* also shows a later, metameric expression pattern in ectodermal and neural cell patches in all trunk segments. Murine homologs of the *Drosophila ems* gene, *Emx1* and *Emx2*, have been cloned on the basis of conservation of the homeobox sequence (Simeone *et al.,* 1992a; Simeone *et al.,* 1992b). Both genes are involved in early embryonic brain development where they show nested expression domains in the developing cerebral cortex and olfactory bulbs. Expression data suggest that the *Emx* genes might be involved in dorsal telencephalic development.

Mutant analysis indicates that *ems* is involved in neurogenesis in embryonic brain development; *ems* loss-of-function leads to a gap-like deletion of the deutocerebral and tritocerebral anlagen of the embryonic *Drosophila* brain (Hirth *et al.,* 1995). To determine if the murine homologs of *ems* are capable of restoring the brain phenotype of *ems* mutant flies, genetic rescue experiments involving ubiquitous overexpression of the mouse *Emx2* gene were carried out (Hartmann *et al.,* 2000). When the *Emx2* transgene was overexpressed in the *ems* null mutant, substantial restoration of brain morphology was observed. Thus, in over one fourth of the cases, the cellular gap in the deutocerebral and tritocerebral anlagen was restored. This suggests that a functional murine *Emx2* gene can replace the *ems* gene to a large degree in the development of the anterior part of the *Drosophila* brain. In the mouse, genetic *in vivo* studies carried out on null mutants for the *Emx* genes show that these genes are necessary for the establishment of discrete regions of the telencephalon (Pellegrini *et al.,* 1996; Yoshida *et al.,* 1997; Qiu *et al.,* 1996). Mutation of *Emx2* leads to a deletion of the dentate gyrus and to a reduction in size of the hippocampus and medial limbic cortex. Mutation of *Emx1* results in the disruption of the corpus callosum and more subtle defects in the forebrain. It will be interesting to see if and to what extent the *Drosophila ems* gene can rescue these defects in the embryonic mouse brain.

Functional Genomics of Embryonic Brain Development: from Genes to Gene Networks

The identification and investigation of specific families of developmental control genes that play central and evolutionarily conserved roles in patterning the embryonic brain in animals as diverse as *Drosophila* and mouse represent important steps towards a comprehensive understanding of the molecular genetic networks involved in brain morphogenesis. The advent and implementation of powerful new genomic technology in these two model systems is currently extending these studies. In *Drosophila*, where full genome sequence is available (Adams *et al.,* 2000), it is already possible to combine extensive manipulative molecular genetic technology and large scale functional genomics with the goal of identifying all of the control genes involved in brain development.

One useful strategy will be to manipulate, genetically and in the developing organism, high-order developmental control genes and genetic switches which regulate specific aspects of brain development, and then use full genome DNA microarrays (gene chips) to identify all of the gene transcripts that are influenced by the corresponding genetic manipulation. Once the entire set of genes that are involved in specific aspects of brain formation are known, manipulative genetics can once again be used to investigate individual gene expression and function *in vivo* and, thus, reconstruct the genetic network that controls these processes. Current experiments in *Drosophila* indicate that the use of large scale microarrays permits the simultaneous identification of hundreds of genes that are regulated by cephalic gap and homeotic genes, and many of these identified genes are likely to represent novel target genes which are part of the genetic network that directs embryonic patterning of the brain (Leemans *et al.,* 2001). Since similar experiments should soon, in principle, also be possible in the mouse, it will be important to use comparative functional genomics to identify similarities and differences in the genes and gene networks that control brain development in *Drosophila* and in the mouse as well as in other relevant genetic systems.

Are the Genetic Mechanisms for Embryonic Brain Patterning Universal?

The recent findings in *Drosophila* and mouse reviewed here indicate an evolutionarily conserved role of homeotic genes and cephalic gap genes in brain development. This is supported by comparative data in other animal groups. Thus, the homeotic genes are found in all metazoa examined and may play a fundamental role in nervous system patterning in all animals. For example, in the urochordate ascidians, *Hox* genes are expressed in specific domains of the visceral ganglion and nerve cord suggesting that regionalized *Hox* gene expression in the CNS is an ancient characteristic of the chordates (Katsuyama *et al.,* 1995; Gionti *et al.,* 1998). In the cephalochordate *Amphioxus*, the colinear correlation of *Hox* gene expression patterns and *Hox* gene chromosomal position holds for the nervous system but not for the somites, implying that the use of *Hox* genes in nervous system regionalization may be more primitive than the use of these genes in mesoderm regionalization (Holland and Garcia-Fernandez, 1996). Genes related to the *otd/Otx* family have also been found in the anterior CNS of all invertebrates examined including animals as primitive as planarians (Umesono *et al.,* 1999). Moreover, comparative studies reveal the existence of *otd/Otx*-related genes in all chordates (Simeone *et al.,* 1992a; Bally-Cuif *et al.,* 1995, Li *et al.,* 1994; Mercier *et al.,* 1995; Pannese *et al.,* 1995) including urochordates (Wada *et al.,* 1996), cephalochordates (Williams and Hol-

land, 1998), and agnates (Ueki *et al.,* 1998), where they are expressed in the rostralmost CNS.

This conservation of developmental control gene action in embryonic brain patterning contrasts dramatically with the diversity of brain structures that have arisen in evolution. One solution to this apparent paradox might be that conserved genes such as the *otd/Otx* genes acquired different roles even while retaining an evolutionary functional equivalence. Indeed, evolutionary modification in copy numbers, expression patterns, protein levels, and cell-type specific post-transcriptional control might represent the most rapid and efficient evolutionary tools for generating new molecular interactions that, in turn, could have contributed to the modification of novel morphogenetic processes. If this is the case, then the architecture of the brain might have been dramatically reorganized during evolution through the modification of the genetic regulatory mechanisms that act on conserved control gene functions.

Acknowledgements

I would like to thank colleagues and members of their labs for helpful discussion. I also thank Robert Williams for permission to use data from the Mouse Brain Atlas Project (http://mickey.utmem.edu/atlas/atlas.html). This work was supported by the Swiss NSF and the EU BIOTECH and INTAS Programs.

References

ACAMPORA, D., AVANTAGGIATO, V., TUORTO, F., BARONE, P., REICHERT, H., FINKELSTEIN, R. and SIMEONE, A. (1998) Murine *Otx1* and *Drosophila otd* genes share conserved genetic functions required in invertebrate and vertebrate brain development. *Development* 125: 1691-1702.

ACAMPORA, D., MAZAN, S., AVANTAGGIATO, V., BARONE, P., TUORTO, F., LALLEMAND, Y., BRULET, P. and SIMEONE, A. (1996). Epilepsy and brain abnormalities in mice lacking the *Otx1* gene. *Nat. Genet.* 14: 218-222.

ACAMPORA, D., MAZAN, S., LALLEMAND, Y., AVANTAGGIATO, V., MAURY, M., SIMEONE, A. and BRULET, P. (1995). Forebrain and midbrain regions are deleted in *Otx2-/-* mutants due to a defective anterior neuroectoderm specification during gastrulation. *Development* 121: 3279-3290.

ADAMS, M. D., CELNIKER, S. E., HOLT, R. A., EVANS, C. A., GOCAYNE, J. D., AMANATIDES, P. G., SCHERER, S. E., LI, P. W., HOSKINS, R. A., GALLE, R. F., *et al.* (2000). The genome sequence of Drosophila melanogaster. *Science* 287: 2185-2195.

ANG, S.-L., JIN, O., RHINN, M., DAIGLE, N., STEVENSON, L. and ROSSANT J. (1996). A 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.

ARENDT, D. and NÜBLER-JUNG, K. (1999) Comparison of early nerve cord development in insects and vertebrates. *Development* 126: 2309-2325.

BALLY-CUIF, L., GULISANO, M., BROCCOLI, V., BONCINELLI, E. (1995). *c-otx2* is expressed in two different phases of gastrulation and is sensitive to retinoic acid treatment in the chick embryo**.** *Mech. Dev.* 49: 49-63.

BRUSCA, R.C. and BRUSCA, G.J. (1990). *Invertebrates.* Sinauer/Sunderland.

CALLAERTS, P. HALDER, G. GEHRING W.J. (1997) Pax-6 in development and evolution. *Annu. Rev. Neurosci.* 20: 483-532.

CAPECCHI, M.R. (1997). The role of *Hox* genes in hindbrain development. In *Molecular and Cellular Approaches to Neural Development* (Ed W. M. Cowan, T. M. Jessell and S. L. Zipursky), Oxford University Press, Oxford, pp.334-355.

CHAN, Y.M. and Jan, Y.N. (1999). Conservation of neurogenic genes and mechanisms. *Curr. Opin. Neurobiol.* 9: 582-588.

COHEN, S.M. and JÜRGENS, G. (1990). Mediation of *Drosophila* head development by gap-like segmentation genes. *Nature* 346: 482-485.

DALTON, D., CHADWOCK, R., MCGINNIS, W. (1989). Expression and embryonic function of *empty spiracles*: a *Drosophila* homeo box gene with two patterning functions on the anterior-posterior axis of the embryo. *Genes Dev* 3: 1940-1956.

DOE, C. Q. and SKEATH, J. B. (1996) Neurogenesis in the insect central nervous system. *Curr. Opin. Neurobiol.* 6: 18-24.

DUBOULE, D. and MORATA, G. (1994) Colinearity and functional hierarchy among genes of the homeotic complexes. *Trends Genet,* 10: 358-364.

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 of the *Drosophila* nervous system and ocellar visual structures. *Genes Dev.* 4: 1516-1527.

GAVALAS, A., STUDER, M., LUMSDEN, A., RIJLI, F. M., KRUMLAUF, R. and CHAMBON, P. (1998) *Hoxa1* and *Hoxb1* synergize in patterning the hindbrain, cranial nerves and second pharyngeal arch. *Development* 125: 1123-1136.

GELLON, G. and MCGINNIS W. (1998). Shaping animal body plans in development and evolution by modulation of Hox expression patterns. *Bioessays*, 20: 116-125.

GIONTI, M., RISTORATORE, F., DI GREGORIO, A., ANIELLO, F., BRANNO, M., DI LAURO, R. (1998). Cihox5, a new Ciona intestinalis Hox-related gene, is involved in regionalization of the spinal cord. *Dev Genes Evol* 207: 515-523.

HANKS, M.C., LOOMIS, C.A., HARRIS, E., TONG, C.-X., ANSON-CARTWRIGHT, L., AUERBACH, A., JOYNER, A. (1998). *Drosophila engrailed* can substitute for mouse *Engrailed1* function in mid-hindbrain, but not limb development. *Development* 125: 4521-4530

HARTMANN, B., HIRTH, F., WALLDORF, U. and REICHERT, H. (2000). Expression, regulation and function of the homeobox gene *empty spiracles* in brain and ventral nerve cord development of *Drosophila*. Mech. Dev.

HIRTH, F., HARTMANN, B. and REICHERT, H. (1998). Homeotic gene action in embryonic brain development of *Drosophila*. *Development* 125: 1579-1589.

HIRTH, F. and REICHERT, H. (1999) Conserved genetic programs in insect and mammalian brain development. *Bioessays* 21: 677-684

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 genes *orthodenticle* and *empty spiracles* in *Drosophila*. *Neuron* 15: 769-778.

HOLLAND, P.W.H, GARCIA-FERNANDEZ, J. (1996). Hox genes in chordate evolution. *Dev. Biol.* 173: 383-395.

JÜRGENS, G. and HARTENSTEIN, V. (1993) The terminal regions of the body pattern. In *The Development of Drosophila* (Ed. M. Bate and A. Martinez-Arias), Cold Spring Harbor Laboratory Press, New York, pp. 687-746.

KATSUYAMA, Y., WADA, S., YASUGI, S., SAIGA, H. (1995). Expression of the labial group gene HrHox-1 and its alteration by retinoic acid in development of the ascidian Halocynthia roretzi. *Development* 121: 3197-3205.

KAUFMAN, T.C., SEEGER, M.A. and OLSEN, G. (1990). Molecular and genetic organization of the antennapedia gene complex of *Drosophila melanogaster. Adv. Genet.* 27: 309-362.

LEE, J.E. (1997). Basic helix-loop-helix genes in neural development. *Curr. Opin. Neurobiol.* 7: 13-20.

LEEMANS, R., LOOP, T., EGGER, B., He, H., KAMMERMEIER, L., HARTMANN, B., CERTA, U., REICHERT, H., and HIRTH, F. (2001). Identification of candidate downstream genes for the homeodomain transcription factor Labial in Drosophila through oligonucleotide-array transcript imaging. *Genome Biol.* 2:research 0015.1-0015.9.

LEUZINGER, S., HIRTH, F., GEHRLICH, D., ACAMPORA, D., SIMEONE, A., GEHRING, W.J., 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.

LI, Y., ALLENDE, M.L., FINKELSTEIN, R., WEINBERG, E.S. (1994). Expression of two zebrafish *orthodenticle*-related genes in the embryonic forebrain. *Mech. Dev.* 48: 229-244.

LUMSDEN, A. and KRUMLAUF, R. (1996). Patterning the vertebrate neuraxis. *Science* 1996, 274: 1109-115.

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.

MCGINNIS, W. and KRUMLAUF, R. (1992) Homeobox genes and axial patterning. *Cell*, 68**:** 283-302.

MERCIER, P., SIMEONE, A., COTELLI, F., BONCINELLI, E. (1995). Expression patterns of two *Otx* genes suggest 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 *in situ* hybridisation and chick/quail homotopic grafts. *Development* 122: 3785-3797.

NAGAO, T., LEUZINGER, S., ACAMPORA, D., SIMEONE, A., FINKELSTEIN, R., REICHERT, H., FURUKUBO-TOKUNAGA, K. (1998). Developmental rescue of *Drosophila* cephalic defects by human *Otx* genes. *Proc. Natl. Acad. Sci. USA* 95: 3737-3742.

NIELSEN, C. (1995). *Animal Evolution. Interrelationships of the Living Phyla.* Oxford University/Oxford.

PANNESE, M., POLO, C., ANDREAZZOLI, M., VIGNALI, R., KABLAR, B., BARSACCHI, G. BONCINELLI, E. (1995). The *Xenopus* homologue of *Otx2* is a maternal homeobox gene that demarcates and specifies anterior structures in frog embryos. *Development* 121: 707-720.

PELLEGRINI, M., MANSOURI, A, SIMEONE, A., BONCINELLI, E., GRUSS, P. (1996). Dentate gyrus formation requires *Emx2. Development* 122: 3893-3898.

REICHERT, H. and BOYAN, G. (1997). Building a brain: developmental insights in insects. *Trends Neurosci.* 20: 258-264.

REICHERT, H. and SIMEONE, A. (1999). Conserved usage of gap and homeotic genes in patterning the CNS. *Curr. Opin. Neurobiol.* 9: 589-595.

RUBENSTJEIN, 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.

RUDDLE, F.H., BARTELS, J.L., BENTLEY, K.L., KAPPEN, C., MURTHA, M.T. and PENDELTON, J.W. (1994). Evolution of *Hox* genes. *Annu. Rev. Genet.* 28: 423-442.

SCHMIDT-OTT, U., GONZALEZ-GAITANG, M., JÄCKLE, H. and TECHNAU, G. (1994) Number, identity, and sequence of the *Drosophila* head segments as revealed by neural elements and their deletion patterns in mutants. *Proc. Natl. Acad. Sci. USA* 91: 8363-8367.

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.

SIEWING, R (1985). *Lehrbuch der Zoologie.* Gustav Fischer/Stuttgart.

SIMEONE, A. (1998). *Otx1* and *Otx2* in the development and evolution of the mammalian brain. *EMBO J.* 17: 6790-6798.

SIMEONE, A., ACAMPORA, D., GULISANO, M., STORNAIUOLO, A. and BONCINELLI, E. (1992a) Nested expression domains of four homeobox genes in the developing rostral brain. *Nature* 358: 687-690.

SIMEONE, A. GULISANO, M., ACAMPORA, D. STORNAIUOLO, A., RAMBALDI, M. 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., MALLAMAC, 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.

STUDER, M., GAVALAS, A., MARSHALL, H., ARIZA-MCNAUGHTON, L., RIJLI, F. M., CHAMBON, P. and KRUMLAUF, R. (1998). Genetic interactions between *Hoxa1* and *Hoxb1* reveal new roles in regulation of early hindbrain patterning. *Development* 125: 1025-1036.

THERIANOS, S., LEUZINGER, S., HIRTH, F., GOODMAN, C. S. and REICHERT, H. (1995) Embryonic development of the *Drosophila* brain: formation of commissural and descending pathways. *Development* 121: 3849-3860.

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

QIU, M., ANDERSON, S., CHEN, S., MENESES, J.J., HEVNER, R., KUWANA, E., PEDERSEN, R.A., RUBENSTEIN, J.L. (1996). Mutation of the *Emx-1* homeobox gene disrupts the corpus callosum. *Dev. Biol.* 178: 174-178.

UEKI, T., KURATANI, S., HIRANO, S., AIZAWA, S. (1998). *Otx* cognates in a lamprey, Lampetra japonica. *Dev. Genes Evol.* 208: 223-228.

UMESONO, Y., WATANABE, K., AGATA, K. (1999). Distinct structural domains in the planarian brain defined by the expression of evolutionarily conserved homeobox genes. *Dev. Genes Evol.* 209: 31-39.

VIEILLE-GROSJEAN, I., HUNT, P., GULISANO, M., BONCINELLI, E. and THOROGOOD, P. (1997) Branchial HOX gene expression and human craniofacial development. *Dev. Biol.* 183: 49-60.

WADA, S. KATSUYAMA, Y., SATO, Y., ITOH, C., SAIGA, H. (1996). *Hroth*, an *orthodenticle*-related homeobox gene of the ascidian, *Halocynthia roretzi:* its expression and putative roles in axis formation during embryogenesis. *Mech. Dev.* 60: 59-71.

WALLDORF, U., GEHRING, W.J. (1992). *Empty spiracles,* a gap gene containing a homeobox involved in *Drosophila* head development. *EMBO J.* 11: 2247-2259.

WILLIAMS, N.A., HOLLAND, P.W.H. (1998). Gene and domain duplication in the chordate *Otx* family: insights from amphioxus *Otx. Mol. Biol. Evol.* 15: 600-607.

YOUNOSSI-HARTENSTEIN, A., GREEN, P., LIAW, G.-W., 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.

YOSHIDA, M., DUDA, Y., MATSUO, I., MIYAMOTO, N., TAKEDA, N., KURATANI, S., AIZAWA, S. (1997). *Emx1* and *Emx2* functions in development of dorsal telencephalon. *Development* 124: 101-111.

YOUNOSSI-HARTENSTEIN, A., NASSIF, C., GREEN, P. and HARTENSTEIN, V. (1996). Early neurogenesis of the *Drosophila* brain. *J. Comp. Neurol.* 370: 313-329.