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REVIEW

Insights into the urbilaterian brain: conserved genetic patterning mechanisms in insect and vertebrate brain development

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Recent molecular genetic analyses of *Drosophila melanogaster* and mouse central nervous system (CNS) development revealed strikingly similar genetic patterning mechanisms in the formation of the insect and vertebrate brain. Thus, in both insects and vertebrates, the correct regionalization and neuronal identity of the anterior brain anlage is controlled by the cephalic gap genes *otd/Otx* and *ems/Emx*, whereas members of the *Hox* genes are involved in patterning of the posterior brain. A third intermediate domain on the anteroposterior axis of the vertebrate and insect brain is characterized by the expression of the *Pax2/5/8* orthologues, suggesting that the tripartite ground plans of the protostome and deuterostome brains share a common evolutionary origin. Furthermore, cross-phylum rescue experiments demonstrate that insect and mammalian members of the *otd/Otx* and *ems/Emx* gene families can functionally replace each other in embryonic brain patterning. Homologous genes involved in dorsoventral regionalization of the CNS in vertebrates and insects show remarkably similar patterning and orientation with respect to the neurogenic region (ventral in insects and dorsal in vertebrates). This supports the notion that a dorsoventral body axis inversion occurred after the separation of protostome and deuterostome lineages in evolution. Taken together, these findings demonstrate conserved genetic patterning mechanisms in insect and vertebrate brain development and suggest a monophyletic origin of the brain in protostome and deuterostome bilaterians.
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Introduction

The question of whether the last common ancestor of bilaterians had an anatomically complex central nervous system (CNS) is controversial. Evidence from the new molecular-based phylogeny implicates the absence of intermediate taxa at the basis of protostome–deuterostome lineage separation (Figure 1) (Adoutte *et al*, 2000). One important consequence is that traits homologous in arthropods and vertebrates must have been present in the common 'urbilaterian' ancestor from which protostomes and deuterostomes diverged. Several attempts to reconstruct the last common bilaterian ancestor and determine the origin of the CNS of organisms as different as insects and vertebrates have been made in the past. Based on differences in embryonic topography and morphogenesis of the nervous system, bilaterian animals have been subdivided into different groups thought to be characterized by the evolutionary independent origin of their nervous systems (eg Brusca and Brusca, 1990). Contrasting with this notion of independent origins is a large amount of molecular genetic data generated in several vertebrate and invertebrate model systems which indicate that key developmental processes, such as proliferation, regionalization, and specification of the embryonic nervous system, are controlled by homologous genes in vertebrates and insects (reviewed in Arendt and Nübler-Jung, 1999; Reichert and Simeone, 1999; Sprecher and Reichert, 2003). Indeed, evidence from recent experiments in *Drosophila melanogaster* (*D. melanogaster*) and mouse indicates that basic genetic mechanisms involved in embryonic brain development are conserved and suggest a common evolutionary origin of the protostome and deuterostome brain. Here we review the basic regulatory mechanisms of brain development in *D. melanogaster* and mouse from a comparative developmental genetic perspective. Recent expression data and functional experiments on key developmental control genes, such as the dorsoventral patterning genes, the cephalic gap genes *otd*/*Otx* and *ems*/*Emx*, or the *Hox* and *Pax*2/5/*8* genes, are reconsidered in the light of a possible common origin of the bilaterian brain.

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Overview of embryogenesis of the brain in insects and vertebrates

The insect brain is composed of an anterior supraesophageal ganglion and a posterior subesophageal ganglion. The supraesophageal ganglion is subdivided into the protocerebrum, the deutocerebrum, and the trito cerebrum, whereas the subesophageal ganglion is subdivided into the mandibular, maxillary, and labial neuromeres (Therianos *et al*, 1995; Younossi-Hartenstein *et al*, 1996; Campos-Ortega and Hartenstein, 1997;

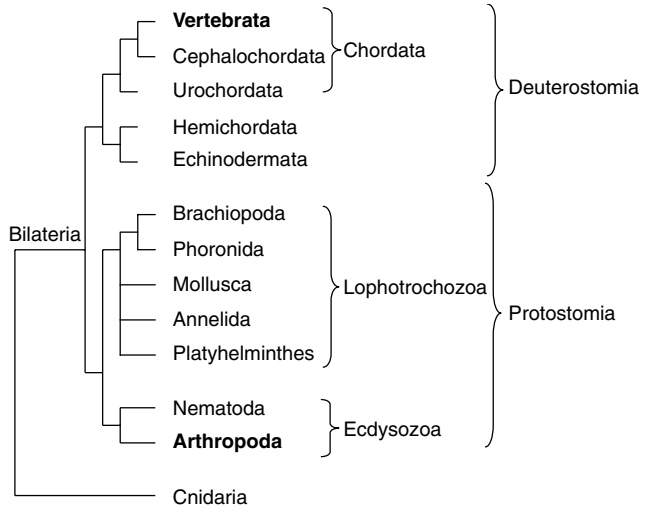

Figure 1 Phylogenetic relationship of mouse and *D. melanogaster*. Simplified version of the new molecular-based phylogeny showing a selection of bilaterian phyla with the Cnidaria as outgroup. Bilaterian phyla are grouped into major cladistic classifications indicated at the right side (modified after Adoutte *et al*, 2000). Vertebrates and arthropods are evidenced in bold. The phylogenetic tree indicates that homologous features of mouse and *D. melanogaster* already existed in the common ancestor of all bilaterian animals.

Reichert and Boyan, 1997). The anterior brain anlage of *D. melanogaster* derives from the procephalic neurogenic region, which is specified to become neuroectoderm through genetic interactions during gastrulation (Jürgens and Hartenstein, 1993). The posterior embryonic brain 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). Within the cephalic neuroectoderm, single progenitor cells called neuroblasts delaminate and start to proliferate, giving rise to the developing brain of *D. melanogaster*.

In vertebrates, inductive interactions between germlayers during gastrulation cause an early regionalization of the developing neural tube. This leads to a rostrocaudal subdivision of the anterior neural tube into the rostral forebrain (prosencephalon or telencephalon/diencephalon) and midbrain (mesencephalon) regions and into the caudal hindbrain regions (rhombencephalon or metencephalon/myelencephalon). The developing hindbrain reveals a clear metameric organization based on seven or eight rhombomeres with pairwise compartment-like organization (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 six neuromeres known as prosomeres (Rubenstein *et al*, 1994, 1998).

Conserved dorsoventral patterning mechanisms indicate a CNS axis inversion in protostome and deuterostome evolution

One of the major arguments during the last two centuries against the common origin of the nervous systems of protostomes and deuterostomes has been the morphologically opposite position of the nerve cords in arthropods (ventral) and vertebrates (dorsal). This striking morphological discrepancy has led to the concept of two taxonomic groups, whose CNS evolved independently from a primitive common ancestor. Invertebrates exhibiting a ventrally located nerve cord such as arthropods, annelids, and mollusks were grouped into the gastroneuralia, whereas the notoneuralia include urochordates, cephalochordates, and vertebrates that are characterized by a dorsal nerve cord (Hatschek, 1891; Brusca and Brusca, 1990). This general notion was first challenged by Geoffroy St Hilaire in the early 19th century who argued, based on morphological considerations, that the ventral side of arthropods corresponds to the dorsal side of vertebrates. Molecular genetic evidence from recent developmental studies in *D. melanogaster* and different vertebrate model organisms have strengthened the view, that the dorsoventral bauplan of protostomes, such as arthropods, represents an inversion of the bauplan of deuterostomes, such as vertebrates. From an evolutionary point of view, this is thought to be the consequence of the inversion of dorsoventral body axis in one of the two animal groups (Arendt and Nübler-Jung, 1994; De Robertis and Sasai, 1996). One of the implications of the dorsoventral inversion theory is that the last common ancestor of protostomes and deuterostomes might already have had a centralized nervous system that was inherited to both descendant lines.

Recent developmental genetic evidence supports the dorsoventral inversion theory at two different levels of neuroectoderm specification (Figure 2). At the level of regionalization of the dorsoventral axis with respect to the presumptive neurogenic region, the early embryos of vertebrates and insects are both patterned by two opposed gradients of homologous morphogens. In accordance with the dorsoventral inversion hypothesis, the transforming growth factor β (TGFβ) family member encoded by the *decapentaplegic* (*dpp*) gene is expressed dorsally in the insect *D. melanogaster*, whereas its vertebrate orthologue *bone morphogenetic protein 4* (*BMP4*) is localized at the ventral side in vertebrates. These factors are antagonized by the secreted products of the homologous genes *short gastrulation* (*sog*) in *D. melanogaster* and *Chordin* in vertebrates (Holley *et al*, 1995; De Robertis and Sasai, 1996; Holley and Ferguson, 1997). The site of action where *sog*/*Chordin* expression inhibits *dpp*/*BMP4* signaling corresponds in fly and mouse to the region of the dorsoventral axis that gives rise to the neuroectoderm in the early embryos. Thus, in insects and vertebrates the antineural function of *dpp*/*BMP4* and the antagonizing neurogenic potential of *sog*/*Chordin* seem to be conserved, whereas their expression gradients are inverted with respect to the dorsoventral body axis.

A second level of dorsoventral patterning of the neuroectoderm has been found to be conserved in evolution as well. A set of homologous genes are involved in the formation of dorsoventral regions of the developing CNS in insects and vertebrates. Again, their relative expression domains are inverted in the sense of a dorsoventral axis inversion between protostomes and deuterostomes (Chan and Jan, 1999; Cornell and Ohlen, 2000). In *D. melanogaster* proneural clusters and early delaminating neuroblasts in the ventral neurectoderm are arranged in three longitudinal columns (medial, intermediate, and lateral) on either

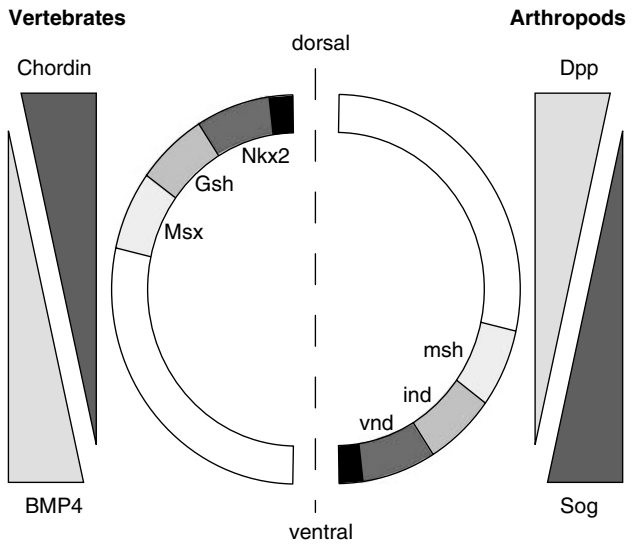

Figure 2 Schematic representation of the molecular genetic patterning of the dorsoventral axis in vertebrates and arthropods. Only half of the body wall is represented for vertebrates and arthropods in the schematic dorsoventral sections with dorsal to the top for both animal groups. The secreted products of the homologous genes *dpp*/*Bmp4* form a dorsoventrally inverted gradient in vertebrates with respect to *D. melanogaster*. They are antagonized by *sog*/*Chordin*, another homologous gene pair, from the region of the embryo that will adopt a neurogenic potential. This region is further patterned by a set of homeobox genes into medial (*vnd*/*Nkx2*), intermediate (*ind*/*Gsh*) and lateral (*msh*/*Msx*) neurogenic domains.

side of the midline cells (reviewed in Skeath and Thor, 2003). Similarly, in vertebrates, such as frog (Chitnis *et al*, 1995) and zebrafish (Haddon *et al*, 1998), proneural clusters that give rise to primary neurons are arranged in three columns on each side of the neural plate (medial, intermediate, and lateral). In *D. melanogaster*, the homeobox genes *ventral nerve cord defective* (*vnd*), *intermediate neuroblasts defective* (*ind*) and *muscle-specific homeobox* (*msh*) are essential for the formation and specification of neuroblasts in the medial, intermediate, and lateral longitudinal columns (Chu *et al*, 1998; McDonald *et al*, 1998; Weiss *et al*, 1998). In the neural plate of vertebrates, the expression of the homologous genes of the *Nkx2* (*vnd*), *Gsh* (*ind*), and *Msx* (*msh*) families defines the medial, intermediate, and lateral neurogenic columns and are involved in their specification (reviewed in Arendt and Nübler-Jung, 1999).

The functional conservation and the similar relative expression patterns of these dorsoventral patterning genes in vertebrates and insects strongly suggest a common origin of the CNS of protostomes and deuterostomes. Accordingly, a reasonable explanation for the opposed positions of the CNS in these two animal groups is the dorsoventral axis inversion between protostomes and deuterostomes. This is further supported by independent molecular evidence from gene expression data in the developing heart of chordates and arthropods. In *D. melanogaster*, the homeobox gene *tinman* is expressed in the dorsal vessel, an insect equivalent of the vertebrate heart (Bodmer, 1993). *Csx* (also called *Nkx2.5*) is a murine orthologue of *tinman*, and is expressed in the ventrally located heart primordium of the mouse embryo (Tanaka *et al*, 1999).

Alternative scenarios for the evolution of centralized nervous systems in protostomes and deuterostomes have been proposed where centralization occurred independently, after the split of the two taxonomic groups, without a dorsoventral inversion (reviewed in Gerhart, 2000; Holland, 2003; Lacalli, 2003). Recently, two hypotheses gained support from molecular genetic studies on hemichordates, a basal deuterostome phylum (Figure 1). In the auricularia hypothesis (Garstang, 1928), the evolutionary origins of the chordate nervous system may be found in the ciliary bands of a deuterostome dipleurula-like larval ancestor. Bilateral ciliary rows and the associated nerves moved dorsally, fused at the midline, and sank inside to form a dorsal cord. A number of genes that are involved in chordate CNS development, including *SoxB3*, *Nkx2.1* and *Otx* are expressed in ciliary bands of larval hemichordates and/or echinoderms (Tagawa *et al*, 2001; Taguchi *et al*, 2002; Takacs *et al*, 2002). So far, however, the ciliary band derivatives have not been shown to give rise to cells of the adult nervous system after metamorphosis. Furthermore, the auricularia hypothesis does not take into account the molecular genetic similarities between the CNS of protostomes and chordates.

A recent comparative study on an enteropneust hemichordate has shown that the anteroposterior expression pattern of a large number of genes, which are involved in axial patterning of the vertebrate and arthropod CNS, is conserved in the diffuse nervous system of the enteropneust worm. The body-encircling basiepithelial nerve net of the directly developing hemichordate, *Saccoglossus kowalevskii*, expresses complex regulatory gene networks in a circumferential way (Lowe *et al*, 2003). Based on the findings in *S. kowalevskii*, Lowe *et al* proposed that the nervous system of the common ancestor of hemichordates, chordates and protostomes was organized in a diffuse, body-encircling, basiepithelial way. A diffuse nervous system is also found in the potentially relevant outgroups to the bilaterian animals, the cnidarians and ctenophores, and could therefore be an ancient condition of Bilateria. According to this view, independent centralization events in chordates and protostomes without dorsoventral inversion could have resulted in anteroposteriorly oriented CNSs with similar gene expression domains. The asymmetric expression along the dorsoventral axis of three genes (*rx*, *nkx2.1*, and *hox4*) described by Lowe *et al* indicates the presence of a dorsoventral patterning program. The antineural mechanism involving *dpp*/*BMP4* that has been shown to limit the nervous systems of *D. melanogaster* and vertebrates to one side of the body is obviously not acting on the nervous system of the enteropneust hemichordate. Therefore, this antineural mechanism would have emerged independently in protostomes and chordates, assuming a noncommon origin of the CNS from a diffusely organized nervous system. This, however, would represent an example of parallel or even convergent evolution and thus not be parsimonious (Gould, 2002). Alternatively, an antineural mechanism along the dorsoventral body axis could have been present in the common ancestor of protostomes and deuterostomes, assuming that the diffuse nervous system of *S. kowalevskii* represents the secondary loss of a CNS and the antineural signaling system. Taken together, the conserved patterning mechanisms giving

rise to a neurogenic region and an opposed antineural region along the dorsoventral axis in arthropods and vertebrates support a common origin of the CNS of protostomes and deuterostomes, including a dorsoventral inversion between the two animal groups.

The homeotic genes pattern the posterior brain in insects and vertebrates

The homeobox or *Hox* genes code for transcription factors with a characteristic helix–turn–helix DNA-binding motif called the homeodomain. Homeotic genes involved in specifying anteroposterior segment identity in the ectoderm were first discovered in *D. melanogaster*. Subsequently, similar clustered homeotic genes were found in a wide range of species where they have been shown to have an essential role in anteroposterior body axis patterning (Ferrier and Holland, 2001; Schilling and Knight, 2001; Carpenter, 2002; Hughes and Kaufman, 2002; Vervoort, 2002). In *D. melanogaster*, the *Hox* genes are arranged in two gene clusters known as the *Antennapedia* (ANT-C) and *Bithorax* (BX-C) complex. The ANT-C contains the five more anteriorly expressed *Hox* genes: *labial (lab)*, *proboscipedia (pb)*, *Deformed (Dfd)*, *Sex combs reduced (Scr)* and *Antennapedia (Antp)*. The BX-C contains the three posteriorly expressed genes: *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*), and *Abdominal-B* (*Abd-B*). Interestingly, the relative position of the genes within the clusters show a correlation with their spatial and temporal expression pattern in the body; genes located towards the 3′ end of the cluster are expressed more anteriorly and earlier in development than genes closer to the 5′ end. This correlation has been termed spatial and temporal colinearity (Mann, 1997). Furthermore, there appears to be a conserved functional hierarchy among the members of the homeotic gene clusters in that more posterior acting genes are functionally dominant over more anterior expressed genes, a fact that has been called 'phenotypic suppression' (Duboule and Morata, 1994). Mammalian *Hox* genes are aligned into 13 paralogous groups which are organized in four chromosomal clusters called *Hox A – Hox D*. The four clusters contain 9–11 *Hox* genes and only the *Hox-B* cluster comprises orthologues of all *D. melanogaster* homeotic complex genes. Similarly, as in *D. melanogaster*, the principle of spatial and temporal colinearity among the paralogous groups is also observed for vertebrate *Hox* genes, and more posterior acting genes impose their developmental specificities upon anterior acting genes what has been termed 'posterior prevalence' (Duboule and Morata, 1994; Mann, 1997).

Hox genes are expressed in the developing CNS of insects and vertebrates in a remarkably similar anteroposterior order (Figure 3a). In *D. melanogaster* genes of the *Hox* clusters are expressed in discrete domains in the developing brain and the ventral nerve cord and their anterior expression boundaries often coincide with neuromere compartment boundaries. In contrast to the embryonic epidermal structures of *D. melanogaster*, the anteroposterior arrangement of the homeotic genes in the fly CNS does not strictly fulfill the criterium of spatial colinearity (Kaufman *et al*, 1990; Hirth *et al*, 1998). The expression domains of the two 3′ most *Hox* genes of the ANT-C are inverted in that the anterior expression

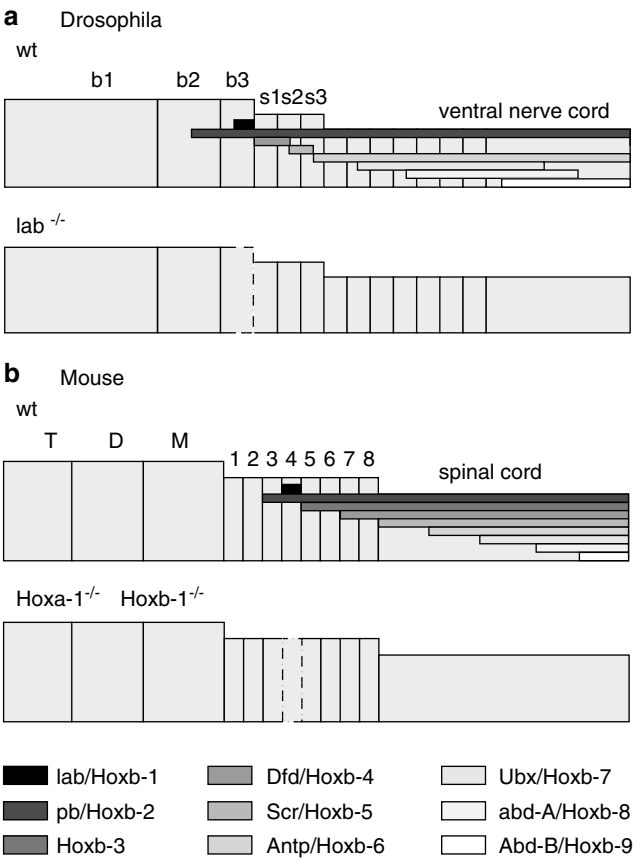

Figure 3 Simplified schematic comparison of *Hox* gene expression domains and mutant phenotypes in the CNS of *D. melanogaster* and mouse. (**a**) Expression domains of the homeotic genes of the Antennapedia and Bithorax complexes in the CNS of *D. melanogaster*: *lab* (*labial*), *pb* (*proboscipedia*), *Dfd* (*Deformed*), *Scr* (*Sex combs reduced*), *Antp* (*Antennapedia*), *Ubx* (*Ultrabithorax*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*). In *lab* null mutant embryos ($lab^{-/-}$), cells of the posterior part of the tritocerebrum (b3) are correctly located in the mutant domain, but fail to assume their correct neuronal cell fate (indicated by dashed lines). (**b**) Expression of the homeotic genes *Hoxb-1*, *Hoxb-2*, *Hoxb-3*, *Hoxb-4*, *Hoxb-5*, *Hoxb-6*, *Hoxb-7*, *Hoxb-8* and *Hoxb-9* in the embryonic CNS of mouse. Double mutant embryos of *Hoxa-1* and *Hoxb-1* ($Hoxa\text{-}1^{-/-}$ $Hoxb\text{-}1^{-/-}$) result in a reduzed size of rhombomere 4 (4) and additionally a loss of expression of rhombomere 4-specific markers (indicated by dashed lines). The synergistic action of *Hoxa-1* and *Hoxb-1* in the specification of rhombomere 4 is comparable to the action of their single orthologue *lab* in the posterior tritocerebrum of *D. melanogaster*. Abbreviations: b1, protocerebrum; b2, deutocerebrum; b3, tritocerebrum; s1, mandibular neuromere; s2, maxillary neuromere; s3, labial neuromere; T, telencephalon; D, diencephalon; M, mesencephalon; 1–8, rhombomeres 1–8; wt, wild type (modified after Hirth and Reichert, 1999).

boundary of *lab* is posterior to that of *pb*. Interestingly, with respect to the relative spatial order of homeotic gene expression, the CNS of *D. melanogaster* is more similar to the CNS of the mouse than to the epidermis of the fly itself. In vertebrates, *Hox* genes are expressed in the hindbrain and spinal cord of the developing CNS. Expression precedes rhombomere formation and becomes progressively restricted to specific domains during embryogenesis. The most anterior *Hox* gene expression in the mouse brain is at the boundary

between rhombomeres 2 and 3. This is followed posteriorly by a set of *Hox* gene expression domains, which generally coincide at their anteriormost domains with rhombomere boundaries. As in the *D. melanogaster* CNS, the mouse orthologues of the *lab* and *pb* genes, *Hoxb-1* and *Hoxb-2*, show an inversion concerning the spatial colinearity rule of *Hox* cluster genes (Figure 3b). This inversion is more likely to have emerged in the CNS of a common ancestor of protostomes and deuterostomes, than independently after the divergence of the two groups.

In *D. melanogaster*, mutational inactivation of either of the *Hox* genes *lab* or *Dfd* results in severe axonal patterning defects in the embryonic brain (Hirth *et al*, 1998). In *lab* null mutants, axonal projection defects occur in the posterior tritocerebrum where *lab* is expressed in the wild-type brain. In the mutant, longitudinal pathways connecting supraesophageal and subesophageal ganglia as well as the projections in the tritocerebral commissure are absent or reduced. Interestingly, the brain defects are not due to a deletion in the tritocerebral neuromere; neuronal progenitors are present and give rise to progeny in the mutant domain. These postmitotic cells, however, do not form axonal and dendritic extensions and are not contacted by axons from other parts of the brain. The *lab* mutant cells do not acquire a neuronal fate, as revealed by the absence of neuronal markers, but rather remain in an undifferentiated state (Figure 3a). Comparable defects are seen in the *D. melanogaster Dfd* mutant in the corresponding mandibular domain, where the wild-type expression of the gene is located. Thus, the appropriate expression of the homeotic genes *lab* and *Dfd* is essential for the establishment of regionalized neuronal identity in the brain of *D. melanogaster*.

In mouse, the *lab* orthologues *Hoxa-1* and *Hoxb-1* are expressed in overlapping domains, with a sharp anterior boundary coinciding with the presumptive rhombomere 3/4 border. Single loss-of-function mutations of particular *Hox* paralogues show marked phenotypic differences suggesting synergy or functional compensation mechanisms (Maconochie *et al*, 1996; Rijli *et al*, 1998; Carpenter, 2002). Functional inactivation of *Hoxa-1* causes segmentation aberrations leading to a reduced size of rhombomeres 4 and 5, defects of motor neuron axonal projections, and malformations of the trigeminal and facial/vestibuloacoustic nerve, but the normal identity of rhombomere 4 is not altered (Gavalas *et al*, 1998; Rijli *et al*, 1998; Studer *et al*, 1998). In contrast, loss of *Hoxb-1* function has no effect on the size of rhombomere 4, but results in a loss of identity of the segment and a partial transformation into a rhombomere 2 identity (Goddard *et al*, 1996; Studer *et al*, 1996). The *Hoxa-1, Hoxb-1* double loss-of-function mutant results in a territory of unknown identity and reduced size between rhombomeres 3 and 5, suggesting a synergistic action of the two genes in rhombomere 4 specification (Figure 3b) (Gavalas *et al*, 1998; Studer *et al*, 1998). Thus, *Hoxa-1* and *Hoxb-1* have very similar roles in the specification of neuronal identity during embryonic brain development as their orthologue *lab* in *D. melanogaster*. The similar functions in addition to similar expression domains of the homologous *Hox* genes in the developing hindbrains and posterior brains of fly and mouse support the idea of a common origin of the CNS.

The *ems/Emx* genes are involved in anterior brain development of *D. melanogaster* and mouse

The *D. melanogaster ems* gene belongs to the cephalic gap genes together with *tailless* (*tll*), *orthodenticle* (*otd*), *buttonhead* (*btd*), and *sloppy paired* (*slp*). At the early blastoderm stage of embryogenesis, the cephalic gap genes are broadly expressed in overlapping anterior stripes where their expression is initially regulated by maternal effect genes (Dalton *et al*, 1989; Finkelstein and Perrimon, 1990; Walldorf and Gehring, 1992). The functional inactivation of any of these genes results in gap-like phenotypes where structures of several head segments are missing (Cohen and Jürgens, 1991; Grossniklaus *et al*, 1994). The cephalic gap genes *tll, otd, ems*, and *btd* have been shown to be essential in early brain development. By the time of neuroblast delamination in the anterior brain, their expression domains become restricted to specific subsets of neural progenitors (Younossi-Hartenstein *et al*, 1997; Urbach and Technau, 2003). Mutational inactivation of a given cephalic gap gene results in the deletion of a specific brain area, indicating the requirement of these genes in early specification of the anterior brain primordium (Hirth *et al*, 1995; Younossi-Hartenstein *et al*, 1997).

The expression domain of the homeodomain transcription factor *ems* in the procephalic neuroectoderm and in the subsequently formed early embryonic brain of *D. melanogaster* comprises two stripes in the anterior parts of the deutocerebral (b2) und tritocerebral (b3) neuromeres (Figure 4a). A reiterated segmental expression pattern is also seen in the ventral nerve cord at later embryonic stages (not shown in Figure 4a). Loss-of-function of the *ems* gene results in a gap-like phenotype in the brain due to the absence of cells in the deutocerebral and anterior tritocerebral neuromeres (Hirth *et al*, 1995; Hartmann *et al*, 2000). In the *ems* mutant domain the expression of the proneural gene *lethal of scute* (*l'sc*) is lost and neuroblasts fail to form (Younossi-Hartenstein *et al*, 1997). This phenotype can be rescued by ubiquitous overexpression of *ems*, which results in proper brain development (Hartmann *et al*, 2000). Thus, *ems* function is required for the specification and formation of the anterior embryonic brain in *D. melanogaster*.

The two mouse orthologues, *Emx1* and *Emx2*, of the *D. melanogaster* cephalic gene *ems*, show largely overlapping expression domains in the developing brain. Whereas *Emx1* mutant mice are postnatal viable and show rather subtle phenotypes restricted to the forebrain, *Emx2* mutant mice die immediately after birth (Pellegrini *et al*, 1996; Qiu *et al*, 1996; Yoshida *et al*, 1997). *Emx2* expression is seen in the germinative neuroepithelium of the presumptive cerebral cortex in the developing forebrain at around embryonic day 9.5. During early corticogenesis, *Emx2* is restricted to the germinative layer in the ventricular zone, where it is expressed in proliferating neuroblasts. Subsequently, *Emx2* expression is also found in Cajal-Retzius cells and the most marginal cortical plate neurons in the marginal zone (Gulisano *et al*, 1996; Pellegrini *et al*, 1996; Mallamaci *et al*, 2000). The anteriormost expression of *Emx2* in the brain is found in the olfactory epithelium, whereas posteriorly

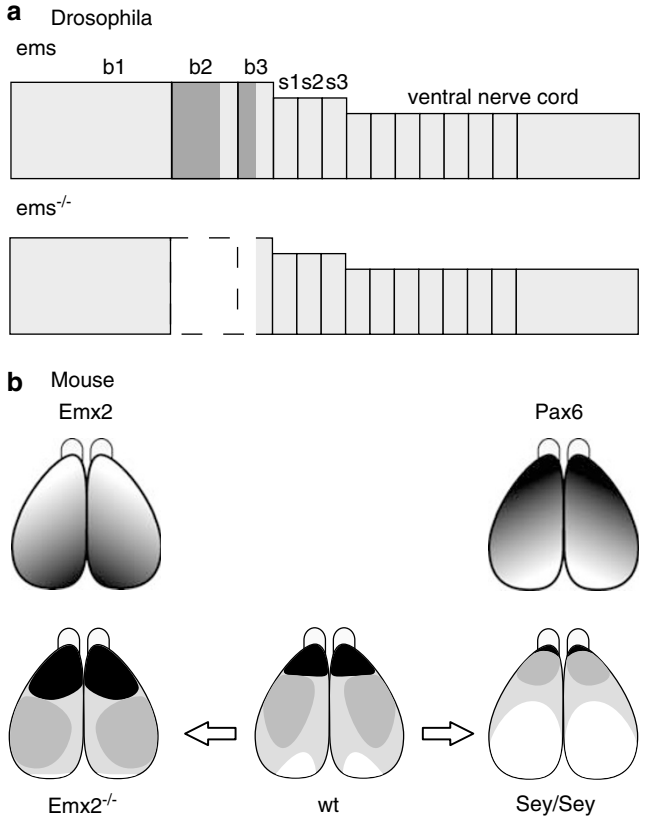

Figure 4 Schematic representation of expression patterns and loss-of-function mutant phenotypes of *ems* in *D. melanogaster* and *Emx2* and *Pax6* in mouse. (**a**) In insects, the *ems* gene is expressed in the anterior part of the deutocerebrum and the anterior part of the tritocerebrum. Mutational inactivation of *ems* (*ems*$^{-/-}$) results in the absence of the deutocerebrum and anterior part of the tritocerebrum. (**b**) In the developing mouse neocortex *Emx2* is expressed in a gradient, with high caudomedial and low rostrolateral expression levels. In *Emx2* null mutants (*Emx2*$^{-/-}$), the anterior motor (black) and sensory (dark gray) cortical areas are expanded caudally, whereas the posterior visual cortical areas (white) are reduced in size. *Pax6* is expressed in a gradient opposite to that of *Emx2* expression in the developing neocortex. An opposite expansion of the cortical areas with respect to *Emx2* mutants is observed in the *Pax6* mutant *Small eye* (*Sey*/*Sey*), which indicates the interaction of *Emx2* and *Pax6* in regionalizing the neocortex (abbreviations, see Figure 3; modified after Hartmann *et al* (2000) and Bishop *et al* (2002)).

the expression domain extends into the roof plate of the diencephalon. *Emx2* is expressed throughout the developing neocortex in a graded manner with high levels at caudomedial and low levels at rostrolateral regions (Figure 4b). An opposed gradient is built up by the *Pax6* gene that has been shown to interact with *Emx2* in the regionalization of the neocortex. Mutational inactivation of *Emx2* results in an expansion of the rostrolateral motor and somatosensory areas at the expense of the caudomedial neocortical areas, such as the visual area. An opposite shift in regional identity is seen in the *Pax6* loss-of-function mutant, while in the *Emx2* and *Pax6* double mutant the cerebral cortex seems to acquire the identity of basal ganglia (Bishop *et al*, 2002; O'Leary and Nakagawa, 2002; Muzio *et al*, 2002). Interestingly, two orthologues of *Pax6*, *eyeless* (*ey*) and *twin of eyeless* (*toy*) are expressed in the anterior brain of the *D. melanogaster* embryo (Kammermeier *et al*, 2001). This raises the question whether they interact with *ems* in the regional specification of the embryonic fly brain.

The expressions of the *D. melanogaster ems* and the mouse *Emx* genes in the developing embryonic brain are similar, as is their ability to confer regional identity to the cells of a specific domain in the brain. Further evidence for the functional equivalence of the *ems* and *Emx2* gene products comes from a cross-phylum rescue experiment carried out in *D. melanogaster* embryos. Ubiquitous overexpression of a mouse *Emx2* transgene in an *ems* null mutant background rescues the brain phenotype of the mutant fly embryos (Hartmann *et al*, 2000).

Functional conservation of *otd/Otx* genes in embryonic brain development of *D. melanogaster* and mouse

The *D. melanogaster* cephalic gap gene *otd* encodes a transcription factor with a *bicoid*-like homeodomain and is required for head development and segmental patterning in the fly embryo. The first *otd* transcripts appear in the anterior region of the early blastoderm stage embryo, where they are expressed in a broad circumferential stripe. During gastrulation this anterior expression domain becomes more and more restricted to the procephalic neuroectoderm, and *otd* is expressed in most delaminating neuroblasts, of the presumptive protocerebrum and anterior deutocerebrum. This corresponds largely to the domain where *otd* is expressed at later embryonic stages in the brain (Hirth *et al*, 1995; Younossi-Hartenstein *et al*, 1997; Urbach and Technau, 2003). Interestingly, *otd* expression is not detected in the anteriormost part of the brain (Figure 5a). A second *otd* expressing domain is found at the ventral midline of the fly embryo in mesectodermal cells that will give rise to neurons and glia of the ventral nerve cord (not shown in Figure 5a). Mutational inactivation of *otd* results in a striking phenotype of the fly embryo in which the entire anterior part of the brain is lacking (Hirth *et al*, 1995). Mutant analysis has shown that most protocerebral and part of the adjacent deutocerebral neuroblasts are absent in the *otd* mutant, a fact that correlates with loss in the expression of the *l'sc* gene, which is thought to be required for neuroectodermal cells to adopt a neuroblast fate (Younossi-Hartenstein *et al*, 1997). In addition to the gap phenotype in the anterior brain, *otd* loss-of-function flies exhibit impairments in the development of visual structures as well as midline defects in the ventral nerve cord (Finkelstein *et al*, 1990). Ubiquitous overexpression of *otd* in a null mutant background at stages preceding neuroblast formation is able to restore anterior brain structures and ventral nerve cord defects. In a wild-type background, ubiquitous overexpression of *otd* results in the generation of ectopic neuronal structures, such as enlarged ganglia. Interestingly, some of the ectopic cells express the protocerebrum-specific gene *brain-specific homeobox* (*bsh*) indicating that *otd* expression might induce a partial protocerebral identity in these neuronal structures (Leuzinger *et al*, 1998).

The two mouse orthologues, *Otx1* and *Otx2*, of the *otd* gene are expressed in nested domains of the developing brain. *Otx1* expression is first observed at approximately 8 days post coitum (dpc) in the neuroepithelium of the

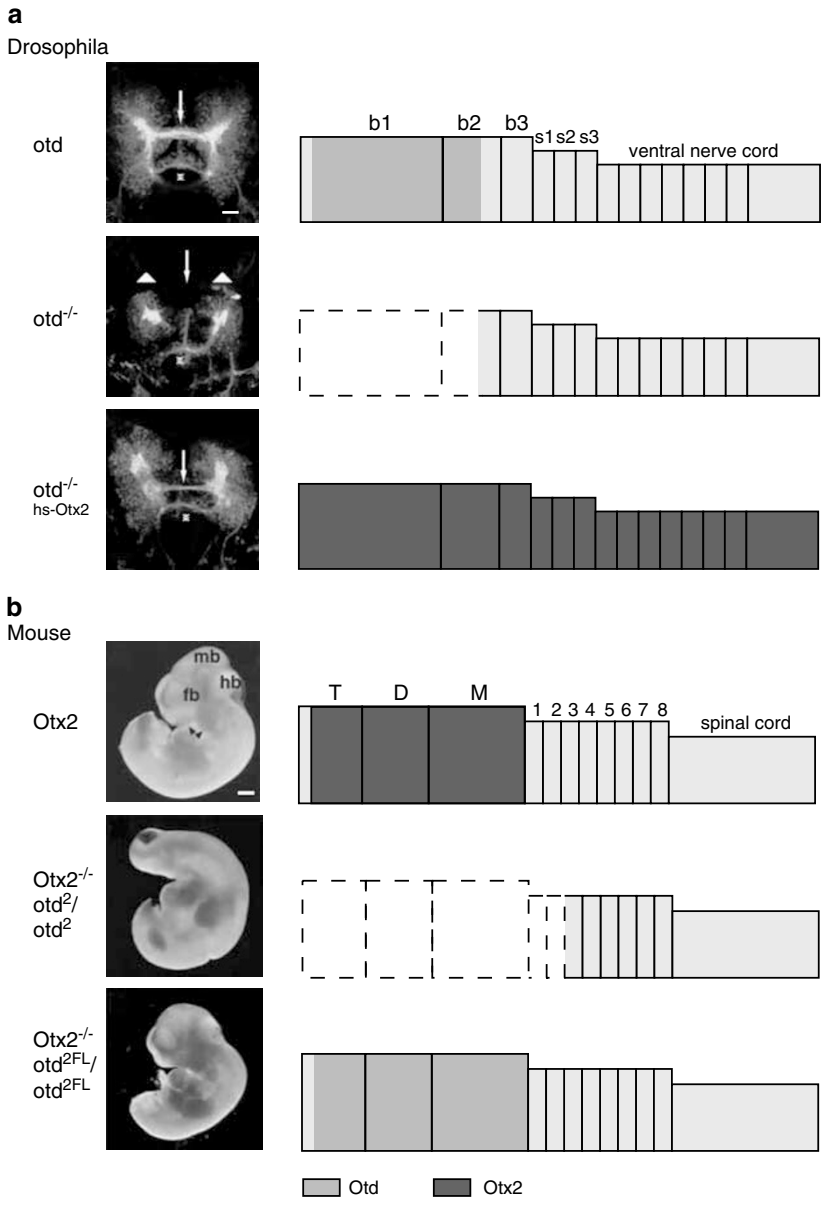

Figure 5 Summary scheme of expression domains, null mutant phenotypes and cross-phylum rescue experiments of the *otd*/*Otx2* genes in *D. melanogaster* and mouse. Genotypic indications on the left of the corresponding rows are indicated in (**a**) and (**b**). The photographs show frontal views of *D. melanogaster* embryonic brains (anti-HRP immunostaining; scale bar: 10 μm) in (**a**) and lateral views of whole mount mouse embryos (embryonic day 10.5; scale bar: 250 μm) in (**b**). The column on the right-hand side shows schematic representations of expression domains and phenotypes in the brain of the corresponding animal and genotype. (**a**) In the fly the *otd* gene is expressed throughout most of the protocerebrum and the anterior part of the deutocerebrum. In the frontal view of the embryonic *D. melanogaster* wild-type brain, the preoral commissure interconnecting the two anterior brain hemispheres is indicated by an arrow (the frontal connective is marked with an asterisk). In *otd* mutant embryos ($otd^{-/-}$), the protocerebrum including the preoral commissure and the anterior deutocerebrum are absent (indicated by triangles in the picture and by dashed lines in the scheme). Overexpression of human *Otx2* gene in *otd* mutant embryos ($otd^{-/-}$; *hs-Otx2*) results in a rescue of the anterior brain including the preoral commissure. (**b**) In mouse the *Otx2* gene is expressed in the anterior part of the embryonic brain including the presumptive telencephalon (except for anteriormost part), diencephalon, and mesencephalon. In the lateral view of the mouse embryo the major brain regions are labelled as forebrain (fb), midbrain (mb), and hindbrain (hb). In *Otx2* null mutants in which the *D. melanogaster otd* replaces the *Otx2* gene ($Otx2^{-/-}$; otd^2/otd^2) the entire forebrain and midbrain (as well as rhombomeres 1 and 2) are absent. In *Otx2* mutants, in which the *otd* coding sequence has been fused to the 3′ and 5′ UTRs of *Otx2* gene ($Otx2^{-/-}$; otd^{2FL}/otd^{2FL}), the hybrid transcript is translated in the anterior neuroectoderm of the mouse embryo and the rostral forebrain is restored (modified after Leuzinger *et al*, 1998; Acampora *et al*, 2001).

presumptive telencephalon, diencephalon, and mesencephalon (Simeone *et al*, 1992). During corticogenesis, *Otx1* expression is maintained in the ventricular zone of the cortical anlage, but decreases as upper layer neurons are generated. By this time, postmigratory neurons of layers 5 and 6 progressively start to express *Otx1*, whereas later differentiated neurons of upper layers 1–4 remain devoid of *Otx1* expression (Frantz *et al*, 1994). *Otx1* is also expressed at early embryonic stages in precursor structures of sense organs, such as the

olfactory epithelium and the inner ear (Simeone *et al*, 1993). *Otx1* null mice are viable but suffer from spontaneous epileptic seizures and exhibit a smaller brain size, mainly due to a reduced thickness of the telencephalic cortex. In addition, the development of the vestibuloacoustic sense organs is impaired, as the lateral semicircular duct of the inner ear is lost (Acampora *et al*, 1996).

The earliest expression of *Otx2* is found in the epiblast and in the visceral endoderm (VE) prior to the onset of gastrulation. During gastrulation, *Otx2* expression is observed in the epiblast and anterior neuroectoderm as well as in the underlaying anterior visceral endoderm (AVE) and the node-derived axial mesendoderm (ame). The AVE and ame are believed to generate *Otx2*-mediated instructive signals that are required in the early specification and patterning of the overlaying anterior neuroectoderm (reviewed in Simeone, 1998; Acampora and Simeone, 1999). *Otx2* expression in the anterior neuroectoderm is maintained during brain regionalization and extends from the telencephalon to the posterior border of the mesencephalon, anterior of the midbrain-hindbrain boundary (MHB) (Figure 5b). Interestingly, the domain of *Otx2* expression does not include the most anterior brain region, which is similar to the expression pattern of *otd* in the embryonic fly brain (Simeone *et al*, 1992).

Otx2 null mice die early in embryogenesis and lack the rostral neuroectoderm fated to become forebrain, midbrain, and rostral hindbrain as a result of an impairment in early specification of the anterior neuroectoderm by the VE (Acampora *et al*, 1995; Rhinn *et al*, 1998). This has been demonstrated in chimeric mouse embryos containing *Otx2*−/− epiblast and wild-type VE, where the early induction of the anterior neural plate was transiently rescued, but subsequent brain development remained impaired. No rescue was obtained in chimeras containing a wild-type epiblast and a *Otx2*−/− VE (Rhinn *et al*, 1998).

Cross-phylum rescue experiments between fly *otd* and mammalian *Otx1* and *Otx2* genes were carried out in order to assess the functional equivalence or diverged properties of the gene homologues. Ubiquitous overexpression of either human *Otx1* or human *Otx2* in an *otd* mutant fly in both cases restored the anterior brain structures absent in the *otd* null mutant (Figure 5a) (Leuzinger *et al*, 1998).

Similar cross-phylum experiments were carried out in mouse with *otd* replacing the vertebrate *Otx* orthologues. In an *Otx1* null mutant background, *otd* is able to fully rescue epilepsy and corticogenesis abnormalities restoring wild-type brain size. However, the lateral semicircular duct of the inner ear is never restored (Acampora *et al*, 1998a). A similar rescue potential is also observed in homozygous mutant mouse embryos, where *Otx1* was replaced with human *Otx2* (Acampora *et al*, 1999; Morsli *et al*, 1999). Thus, the ability to specify the lateral semicircular duct of the inner ear may be an *Otx1*-specific property (Acampora and Simeone, 1999). Gene replacement experiments where different portions of the *Otx2* locus were exchanged with the cDNA of the fly *otd* or human *Otx1* genes revealed a crucial role of regulatory control mechanisms in *Otx2*-specific action during anterior neuroectoderm specification. Two different replacement strategies were utilized. A first mouse model (otd^2/otd^2) was generated in which an *Otx2* region including 5′ and 3′ untranslated regions (UTRs) was replaced with the fly *otd* cDNA, whereas in a second mutant (otd^{2FL}/otd^{2FL}) the *otd* coding sequence was directly fused to the intact 5′ and 3′ UTRs of *Otx2*. In the otd^2/otd^2 mouse model, *otd* is able to take over the early function of the *Otx2* gene in the AVE, leading to a transient restoration of the anterior neural plate absent in *Otx2* mutants. However, otd^2/otd^2 mutants fail to maintain the anterior identities of the neuroectoderm, giving rise to a headless phenotype (Figure 5b). Mutant analysis revealed that *D. melanogaster otd* transcripts were present in both AVE and presumptive anterior neuroectoderm, whereas translation only occurred in the AVE. Additional evidence from similar experiments where *Otx2* including UTRs was replaced with human *Otx1* favored the view that post-transcriptional control was involved in the cell type-specific translation of *Otx2* mRNA in the epiblast and anterior neuroectoderm (Acampora *et al*, 1998b; Boyl *et al*, 2001). This was confirmed in the second mouse model otd^{2FL}/otd^{2FL}, where translation of the hybrid transcript consisting of the fly *otd* fused to the 5′ and 3′ UTRs of *Otx2* occurred in the epiblast and anterior neuroectoderm. Moreover, the correct translation of *otd* in the epiblast and anterior neuroectoderm restored the maintenance of anterior brain pattening in *Otx2* null mutants including the normal positioning of the MHB (Figure 5b) (Acampora *et al*, 2001). This was also shown by a similar hybrid mouse model where human *Otx1* was fused to the 5′ and 3′ UTRs in the mouse *Otx2* locus (Acampora *et al*, 2003). Taken together, *Otx1*, *Otx2*, and *otd* genes show a high degree of functional equivalence in the regions of the developing organism where they are normally expressed. This supports the idea that *otd*/*Otx* functions were originally established in a common ancestor of fly and mouse and conserved throughout evolution. On the other hand, their regulatory control mechanisms appear to have been modified during evolution, thus, generating the specific properties of the genes.

Evidence for a common tripartite ground-plan of the bilaterian brain

A detailed comparison of gene expression patterns and developmental neuroanatomy in vertebrates and urochordates (ascidians) has uncovered a common tripartite ground-plan along the anteroposterior axis for the embryonic CNS. In all cases studied, a rostral brain region expressing *Otx* family genes (corresponding to the vertebrate forebrain and midbrain) is followed by a central region expressing *Pax2/5/8* genes (delimiting the MHB of vertebrates), and subsequently a *Hox* gene expressing caudal region (hindbrain and spinal cord of vertebrates) (Wada *et al*, 1998; Holland and Holland, 1999; Wada and Satoh, 2001). Recently, a similar tripartite pattern of gene expression has been reported for arthropods (see below) and hemichordates, suggesting an evolutionarily more ancient origin of the tripartite organization of brains than chordates (Figure 6a–e) (Hirth *et al*, 2003; Lowe *et al*, 2003; CJ Lowe, personal communication). (Interestingly, no *Pax2/5/8* expression can be detected between the anterior *Otx* and posterior *Hox* expression domains in the neural tube of the cephalochordate *Amphioxus*; the most parsimonious

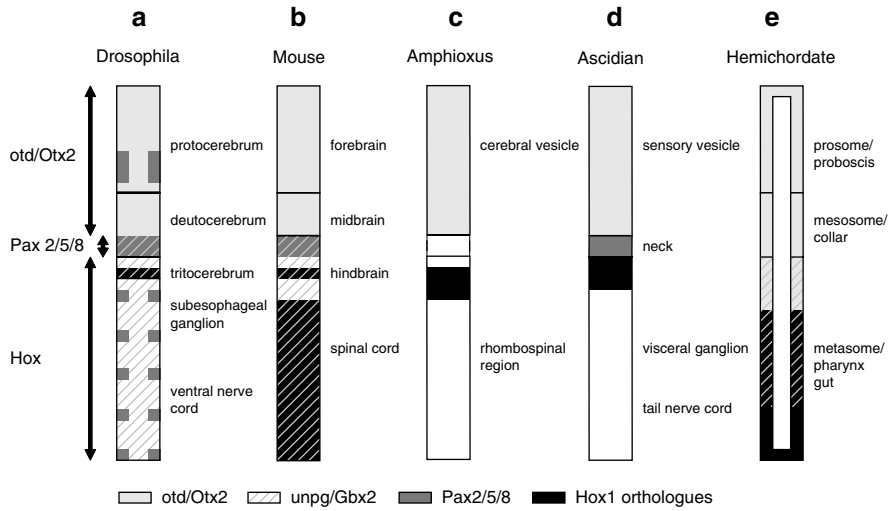

Figure 6 Tripartite ground-plan of the bilaterian nervous system based on expression patterns of orthologous genes in *Drosophila*, mouse, *Amphioxus*, ascidian, and hemichordate. The expression of *otd*/*Otx2*, *unpg*/*Gbx2*, *Pax2*/*5*/*8*, and *Hox1* gene orthlogues in the developing nervous systems of (**a**) stage 13/14 *D. melanogaster* embryo (Hirth *et al*, 2003), (**b**) embryonic day 10 mouse embryo (Wurst and Bally-Cuif, 2001), (**c**) 10 somite stage *Amphioxus* embryo (Wada and Satoh, 2001), (**d**) neurula ascidian (Wada *et al*, 1998) and (**e**) 1 gill slit stage hemichordate embryo (Lowe *et al*, 2003). In all cases an *otd*/*Otx2*-expressing region is located anterior to a *Hox*-expressing region in the posterior nervous system. In *D. melanogaster* and mouse, a *Pax2*/*5*/*8*-expressing domain is positioned at the interface between the anterior *otd*/*Otx2* and the posteriorly abutting *unpg*/*Gbx2* expression domains. In *D. melanogaster*, the *Pax2*/*5*/*8* orthologues *Pax2* and *Poxn* also show a segmentally reiterated expression pattern (see text for details). Up to now, no *unpg*/*Gbx2* orthologues have been isolated in *Amphioxus* and Ascidians. The expression domains of the hemichordate *otd*/*Otx2* and *unpg*/*Gbx2* orthologues show no sharp boundary, but overlap in an intermediate region of the basiepithelial nerve net. Nevertheless, the expression of the hemichordate *Pax2*/*5*/*8* orthologue is consistent with its relative location in chordates (CJ Lowe, personal communication). No *Pax2*/*5*/*8* expression can be found between the *otd*/*Otx2* domain and the *Hox1* domain in *Amphioxus*, which is thought to be due to a secondary reduction.

explanation for this is the secondary loss of the tripartite pattern in the *Amphioxus* CNS (Kozmik *et al*, 1999; Takahashi and Holland, 2004).)

In vertebrate brain development, the *Pax2*/*5*/*8* domain at the MHB is an early marker for the isthmic organizer (IsO), which controls both the growth and the ordered rostrocaudal specification of mesencephalic and metencephalic territories (reviewed by Liu and Joyner, 2001; Rhinn and Brand, 2001; Wurst and Bally-Cuif, 2001). The IsO was first identified through transplantation experiments, in which MHB tissue grafts were transplanted to more rostral or caudal neural locations, resulting in the induction of mesencephalic–metencephalic fate in the host tissue surrounding the graft (Martinez *et al*, 1991; Marin and Puelles, 1994). This organizer-like activity on the surrounding neural tissue is thought to be mediated by fibroblast growth factor 8 (Fgf8) and Wnt1 proteins which are secreted from the MHB tissue. During late gastrulation and early neural plate stages of the vertebrate embryo, the two homeodomain transcription factors *Otx2* and *Gbx2* are expressed in a complementary, mutually exclusive fashion anterior and posterior to the MHB. Whereas *Otx2* null mutant mice lack the brain rostral to rhombomere 3 (see above), mice of the genotype *Otx1*−/− *Otx2*+/− show a rostral extension of metencephalic tissue and the absence of the mesencephalon and caudal diencephalon. Furthermore, the expressions of MHB-specific markers, such as *Fgf8*, *Gbx2*, and *Wnt1*, align in a domain that is shifted rostrally to the corresponding position of prosomere 2 (Acampora *et al*, 1997). Conversely, a caudal shift of MHB markers can be observed in *Gbx2* null mutants, where isthmic nuclei, cerebellum, and rhombomeres 1–3 of the hindbrain are absent (Figure 7a) (Wassarman *et al*, 1997; Millet *et al*, 1999). Together with evidence from misexpression experiments, these results suggest that an antagonistic interaction between *Gbx2* and *Otx2* during early embryonic stages is responsible for the correct positioning of the MHB at their common interface.

Gene expression studies indicate that embryonic anteroposterior patterning of the *D. melanogaster* brain is strikingly similar to the tripartite ground-plan of the vertebrate brain. Expression of both *D. melanogaster Pax2*/*5*/*8* orthologues, *Pox neuro* (*Poxn*) and *Pax2*, is present at the interface of *otd* and the *Gbx2* orthologue *unplugged* (*unpg*), anterior to a *Hox*-expressing region (Noll, 1993; Fu and Noll, 1997; Hirth *et al*, 2003). The expression domains of *Poxn* and *Pax2* span the whole embryonic CNS in a segmentally reiterated pattern, but the genes are never coexpressed in the same cells. Interestingly, the only anteroposterior position along the neuraxis where *Pax2* and *Poxn* are expressed in adjacent domains is located immediately anterior to the deutocerebral–tritocerebral boundary (DTB). In addition, this transversal domain of adjacent *Pax2* and *Poxn* expression differs from the segmentally reiterated expression in more posterior regions by the fact that it is the only position along the neuraxis where expression of both genes coincides with a neuromere boundary (Figure 6a) (Hirth *et al*, 2003). Mutational inactivation of *otd* results in the deletion of the anterior brain of the fly embryo (see above) as well as in the rostral extension of the *unpg* expression domain. In *unpg* loss-of-function mutants, the posterior limit of the anterior brain-specific *otd* expression shifts caudally (Figure 7b). Thus, in both *D. melanogaster* and mouse, mutational inactivation of

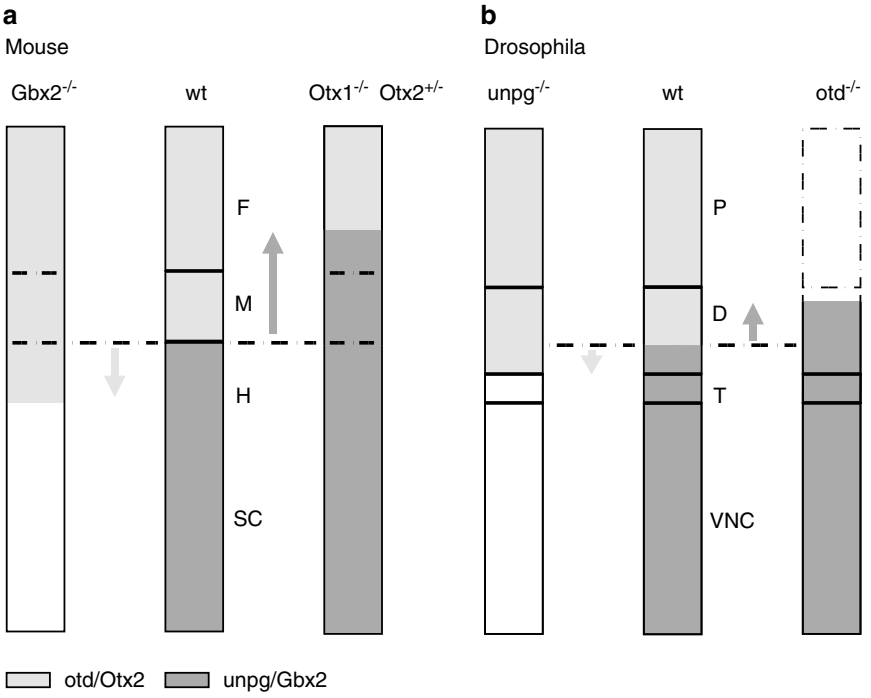

Figure 7 Antagonistic interactions of the *otd*/*Otx* and *unpg*/*Gbx2* genes in the positioning of their common interface. (**a**) Expression domains of *Otx2* and *Gbx2* in the developing mouse CNS corresponding to the six-somite stage in the *Gbx2* null mutant (*Gbx2*$^{-/-}$), wild type (wt), and *Otx1*$^{-/-}$ *Otx2*$^{+/-}$ (*Otx1*$^{-/-}$ *Otx2*$^{+/-}$) genetic background. In the wild-type mouse embryo, *Otx2* is expressed with a sharp limit at the posterior mesencephalon and *Gbx2* expression abuts the *Otx2* expression domain, creating a common interface. In mice homozygous mutant for *Otx1* and heterozygous mutant for *Otx2* (*Otx1*$^{-/-}$ *Otx2*$^{+/-}$), the common interface is shifted anteriorly into the forebrain (dark gray arrow). A posterior expansion of the *Otx2* expression into the hindbrain is observed in *Gbx2* null mutant (*Gbx2*$^{-/-}$) brains. (**b**) Expression domains of *otd* and *unpg* in the developing CNS of *D. melanogaster* in the *unpg* null mutant (*unpg*$^{-/-}$), wild type (wt), and *otd* null mutant (*otd*$^{-/-}$) genetic background. The expression domains of *otd* and *unpg* in the wild-type *D. melanogaster* CNS form a sharp common boundary in the posterior deutocerebrum. In the *otd* null mutant embryo (*otd*$^{-/-}$), the protocerebrum and the anterior deutocerebrum are absent (dashed lines). In addition, the *unpg* expression is shifted anteriorly (dark gray arrow). In the brain of the *unpg* null mutant embryo (*unpg*$^{-/-}$), the *otd*-expressing domain expands posteriorly (light gray arrow). Abbreviations: P, protocerebrum; D, deutocerebrum; T, tritocerebrum; VNC, ventral nerve cord; F, forebrain; M, midbrain; H, hindbrain; SC, spinal cord (modified after Hirth *et al*, 2003; Joyner *et al*, 2000).

otd/*Otx2* and *unpg*/*Gbx2* genes results in the loss or misplacement of an intermediate brain domain characterized by the *otd*/*Otx2* and *unpg*/*Gbx2* interface and by the expression of *Pax2*/*5*/*8* genes. Moreover, *otd*/*Otx2* and *unpg*/*Gbx2* appear to negatively regulate each other at the interface of their expression domains in insects and vertebrates. (Interestingly, *D. melanogaster otd* is able to replace *Otx* gene function in the correct positioning of the MHB during mouse brain development as demonstrated in cross-phylum rescue experiments (see above) (Acampora *et al*, 2001).) Taken together, these results reveal remarkable similarities in gene expression and functional interactions involved in establishing the insect DTB and mouse MHB. However, not all functional interactions among genes involved in MHB formation in the mouse appear to be conserved at the DTB of *D. melanogaster*. Although expression of patterning genes that characterize the vertebrate MHB region, such as *engrailed* (*en*), *Pax2*, *Poxn* or the fly Fgf orthologue *branchless* (*bnl*) can be found at the DTB, no brain-patterning defects are observed in the corresponding null mutant embryos in the fly (Hirth *et al*, 2003). Moreover, even though *D. melanogaster* has a tripartite ground-plan for the developing brain and a boundary region genetically corresponding to the vertebrate MHB, evidence for organizer activity of the fly DTB has not been obtained.

In summary, current comparative data suggest that a tripartite ground-plan for the developing brain was already present in the common ancestor of bilateria. To date, organizer activity of the intermediate boundary region has only been demonstrated in vertebrates (Takahashi and Holland, 2004). As proposed by Wada and Satoh (2001), it may be useful to distinguish between the homology of two characteristics of the vertebrate MHB: homology as a developmental genetic region of the brain and homology as an organizer. In this sense, the *D. melanogaster* DTB can be considered as a region homologous to the vertebrate MHB.

Conclusions

Recent investigations on mechanisms controlling insect and vertebrate brain development have revealed an expanding number of homologous genes with similar expression patterns and comparable functions. The expression and interactions of homologous dorsoventral patterning genes show comparable relative patterning and orientation with respect to the presumptive neurogenic region. Genes of the *otd*/*Otx* and *ems*/*Emx* families are required for correct formation and specification of the developing anterior brain, and *Hox* genes are involved in patterning and specification of the developing posterior brain. Moreover, *otd*/*Otx* genes and *unpg*/*Gbx2* genes

position an intermediate domain between an anterior and a posterior brain region and thus contribute to the tripartite ground-plan of the insect and vertebrate brain.

Taken together, these results imply the evolutionary conservation of genetic programs underlying embryonic brain development in insects and vertebrates. Moreover, the similarities among orthologue groups are not restricted to the positions of the expression domains, but also include functional features. This supports the idea that the protostome and deuterostome brain is homologous in a developmental genetic sense, and thus the common urbilaterian ancestor already had a complex CNS (Arendt and Nübler-Jung, 1996; De Robertis and Sasai, 1996; Hirth and Reichert, 1999). The identification of downstream targets of conserved developmental control genes as well as the analysis of genetic mechanisms at more advanced stages of development should give a deeper insight into the degree of conservation of genetic programs between insects and vertebrates. Specific gene functions that are not shared between orthologous control genes in fly and mouse, such as the post-transcriptional control of the *Otx2* gene in the mouse epiblast, appear primarily to involve modifications of regulatory control elements, but not the coding sequence of the gene. This suggests that genes involved in essential mechanisms of brain development could exert additional, novel functions by modification of their spatial or temporal regulatory control (Acampora and Simeone, 1999; Acampora *et al*, 2001).

A recent gene expression study on hemichordates has led to the view that the deuterostome ancestor might have been characterized by a diffuse basiepithelial nervous system and that a centralized brain could have evolved independently in the deuterostome and protostome lineages (Holland, 2003; Lacalli, 2003; Lowe *et al*, 2003). Homologies in embryonic brain development of vertebrates and insects would therefore derive from axis-patterning mechanisms or the correlating gene expression patterns, which were present in the circumferential nerve net of the last common ancestor. Other similarities that have not been inherited from a common ancestor characterized by a well-patterned nerve net would therefore be a product of convergent or parallel evolution (Gould, 2002). According to this view, the similar antineural function of *dpp/BMP4* in insects and vertebrates represents an example of parallel evolution, assuming that the last common ancestor of protostomes and deuterostomes had a diffuse, body-encircling basiepithelial nervous system. An alternative explanation for the absence of a centralized nervous system in hemichordates is a secondary loss of a CNS together with the antineural activity of the dorsoventral signaling program and the retention of a peripherally located nerve net (Holland, 2003). The expression patterns of the hemichordate orthologues of dorsoventral patterning genes should nurture the discussion on the urbilaterian nervous system.

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