CHAPTER 2

Anteroposterior Regionalization of the Brain: Genetic and Comparative Aspects

Robert Lichtneckert* and Heinrich Reichert

Abstract

Developmental genetic analyses of embryonic CNS development in *Drosophila* have uncovered the role of key, high-order developmental control genes in anteroposterior regionalization of the brain. The gene families that have been characterized include the *otd/Otx* and *ems/Emx* genes which are involved in specification of the anterior brain, the *Hox* genes which are involved in the differentiation of the posterior brain and the *Pax* genes which are involved in the development of the anterior/posterior brain boundary zone. Taken together with work on the genetic control of mammalian CNS development, these findings indicate that all three gene sets have evolutionarily conserved roles in brain development, revealing a surprising evolutionary conservation in the molecular mechanisms of brain regionalization.

Introduction

In most animals, the central nervous system (CNS) is characterized by bilateral symmetry and by an elongated anteroposterior axis, both of which are established very early in embryonic development. During embryogenesis, regionalized anatomical subdivisions appear along the anteroposterior axis, also referred to as the neuraxis. These subdivisions are most prominent near the anterior pole, where the complex structures that comprise the brain are generated. As the brain differentiates, the neuraxis often bends and species-specific flexures arise, which in later stages tend to distort the original anteroposterior coordinates of the CNS. However, when this is taken into account and the neuraxis is reconstructed, remarkable similarities in anteroposterior regionalization of the CNS in animals as diverse as arthropods and vertebrates become apparent. A full appreciation of these similarities comes from combined comparative neuroanatomical and molecular genetic studies carried out in *Drosophila* and mouse, which reveal that comparable, evolutionarily conserved developmental patterning mechanisms operate in regionalization of the embryonic CNS.[1,2]

Here we review recent findings on the developmental genetic control of anteroposterior regionalization in the embryonic CNS in *Drosophila* and compare these findings with investigations carried out on regionalization of the embryonic murine CNS. The similarities in the expression patterns of key developmental control genes together with the comparable functions of these genes during CNS development in flies and mice suggest a common evolutionary origin of the mechanism of embryonic CNS regionalization. Given the current molecular-based phylogeny of bilaterian animals, it seems likely that these features of brain development in arthropods and vertebrates were already present in the common bilaterian ancestor from which protostomes and deuterostomes evolved (Fig. 1).[3] This, in turn, challenges the classical view of an independent origin of protostome and deuterostome brains.

*Corresponding Author: Robert Lichtneckert—Biozentrum, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland. Email: robert.lichtneckert@stud.unibas.ch

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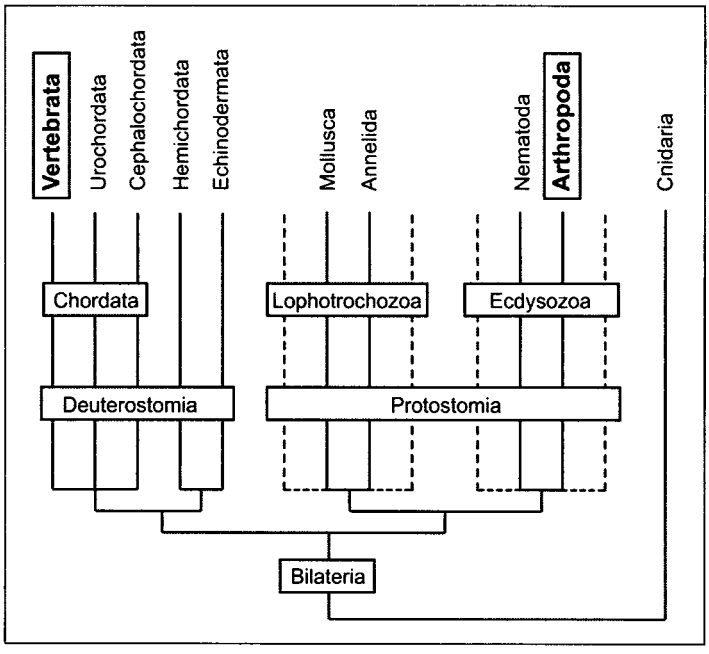

Figure 1. Phylogenetic relationship of Bilateria. Simplified version of the new molecular-based phylogeny showing a selection of bilaterian phyla with the Cnidaria as outgroup. Bilaterian phyla are grouped according to major cladistic classifications. The phylogenetic tree suggests that evolutionarily conserved, homologous features of mouse and *D. melanogaster* already existed in the common ancestor of all bilaterian animals.

The early embryonic CNS of both insects and vertebrates is composed of longitudinally arranged subdivisions that can be grouped into two major parts, an anterior cephalized brain which rapidly forms prominent morphological specializations and a posterior nerve cord-like structure. In insects, the embryonic brain consists of a supraesophageal ganglion that can be subdivided into the protocerebral (b1), deutocerebral (b2) and tritocerebral (b3) neuromeres and a subesophageal ganglion that is subdivided into the mandibular (s1), maxillary (s2) and labial (s3) neuromeres (Fig. 2A). The neuromeres of the developing ventral nerve cord extend posteriorly from the subesophageal ganglion into the body trunk.[4] In vertebrates, the anterior CNS develops three embryological brain regions; the prosencephalon or forebrain (presumptive telencephalon and diencephalon), the mesencephalon or midbrain and the rhombencephalon or hindbrain. The developing hindbrain reveals a metameric organization based on eight rhombomeres and parts of the developing forebrain may also be metamerically organized.[5,6] The developing spinal cord extends posteriorly from the hindbrain into the body trunk.

The topology of these embryonic neuroanatomical regions is reflected in the regionalized expression along the neuraxis of key developmental control genes which appears to be largely conserved between insects and vertebrates. Thus, the anterior CNS of *Drosophila* and mouse is characterized by the expression of the genes *orthodenticle* (*otd/Otx*) and *empty spiracles* (*ems/Emx*). Similarly, the posterior CNS of both species exhibits a conserved and highly ordered expression pattern of the homeotic (*Hox*) gene family. Finally, expression of the *Pax2/5/8* genes defines a third CNS region between the anterior *otd/Otx* and the posterior *Hox* domains, thus revealing a tripartite ground plan of embryonic CNS development in both vertebrates and insects. In the following we consider the roles of each of these three sets of developmental control genes in anteroposterior regionalization of the CNS.

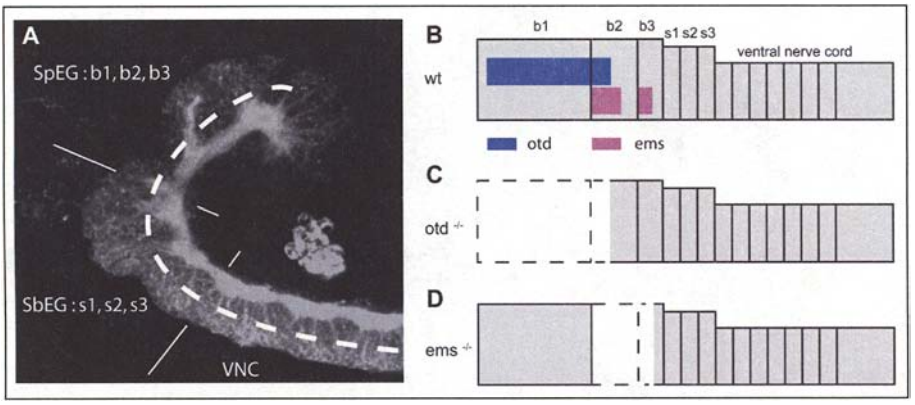

Figure 2. Schematic representation of expression patterns and mutant phenotypes of *otd* and *ems* in the embryonic CNS of *Drosophila*. A) Lateral view of the anterior portion of the embryonic CNS. Because of morphogenetic processes, such as the beginning of head involution, the neuraxis (dashed line) of the embryonic brain curves dorsoposteriorly withing the embryo. Accordingly, in the following, anteroposterior coordinates refer to the neuraxis rather than the embryonic body axis. The major anteroposterior CNS regions are subdivided by white lines. B-D) Schematic representations of the embryonic brain with anterior towards the left and posterior towards the right. B) In the wild type (wt) brain the *otd* gene is expressed throughout most of the protocerebrum (b1) and the anterior part of the deutocerebrum (b2). Expression of *ems* in the brain is restricted to the anterior part of the deutocerebrum and the anterior part of the tritocerebrum (b3). The segmentally reiterated expression patterns of both *otd* and *ems* are omitted for clarity in this schematic. C) In *otd* mutant embryos ($otd^{-/-}$) the protocerebrum and the anterior deutocerebrum are absent (indicated by dashed lines). D) Mutational inactivation of *ems* ($ems^{-/-}$) results in the absence of the deutocerebrum and anterior part of the tritocerebrum. Abbreviations: b1, protocerebrum; b2, deutocerebrum; b3, tritocerebrum; s1, mandibular neuromere; s2, maxillary neuromere; s3, labial neuromere; SbEG, subesophageal ganglion; SpEG, supraesophageal ganglion; VNC, ventral nerve cord.

The Cephalic Gap Genes *Otd/Otx* and *Ems/Emx* Control Anterior Brain Development

The *orthodenticle* (*otd*) and *empty spiracles* (*ems*) homeobox genes belong to the cephalic gap genes in *Drosophila* together with *tailless* (*tll*), *buttonhead* (*btd*) and *sloppy paired* (*slp*). At the early blastoderm stage of embryogenesis, the cephalic gap genes are broadly expressed in overlapping anterior domains under the control of maternal genes.[7-9] The functional inactivation of any of these transcription factors results in gap-like phenotypes where structures of several head segments are missing.[10,11] In addition, the cephalic gap genes *tll*, *otd*, *ems* and *btd* have been shown to play essential roles in early brain development. By the time of neuroblast delamination, their expression domains become restricted to specific subsets of neural progenitors in the anterior procephalic neuroectoderm.[12,13] 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.[13,14]

The 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. In the early blastoderm stage embryo, *otd* is first expressed in a broad circumferential stripe in the anterior region. During gastrulation, however, expression becomes more and more restricted to the anterior procephalic neuroectoderm, where *otd* is expressed in most delaminating neuroblasts of the presumptive protocerebrum (b1) and anterior deutocerebrum (b2).[12,13] This expression domain corresponds largely to the *otd* expression pattern detected at later embryonic stages in the brain[14]

(Fig. 2B). Interestingly, *otd* expression is not observed in the anterior most protocerebral region. An additional, segmentally reiterated expression pattern of *otd* 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 Fig. 2B). Comparable to *otd*, the homeobox gene *ems* is first expressed in a broad stripe posterior and adjacent to *otd* in the early blastoderm stage embryo. In the procephalic neuroectoderm and in the subsequently formed early embryonic brain *ems* expression becomes restricted to two stripes in the anterior parts of the deutocerebral (b2) and tritocerebral (b3) neuromeres (Fig. 2B). In the ventral nerve cord *ems* expression is also found in a segmentally repeated pattern (not shown in Fig. 2B).[14,15]

Mutational inactivation of either *otd* or *ems* results in striking embryonic brain phenotypes in which large brain regions are absent. In the *otd* mutant the entire anterior part of the brain is lacking (Fig. 2C) and mutant analysis has shown that most protocerebral neuroblasts and part of the adjacent deutocerebral neuroblasts are absent in the procephalic neuroectoderm.[13,14] In addition to the gap phenotype in the anterior brain, *otd* mutant flies exhibit impairments in the development of visual structures as well as midline defects in the ventral nerve cord.[8] Ubiquitous overexpression of *otd* in a null mutant background at specific stages preceding neuroblast formation is able to restore anterior brain structures and ventral nerve cord defects.[16] Similarly, loss-of-function of the *ems* gene results in a gap-like phenotype in the embryonic brain due to the absence of cells in the deutocerebral and tritocerebral neuromeres (Fig. 2D). Additionally, axon pathfinding defects can be observed in the ventral nerve cord of *ems* mutant embryos. These phenotypes are rescued by ubiquitous overexpression of *ems* during specific early embryonic stages.[15] Mutant analysis for both *otd* and *ems* shows that the absence of cephalic gap gene expression in the procephalic neuroectoderm correlates with the loss in the expression of the proneural gene *lethal of scute* (*l'sc*) and the ability to form neuroblasts in the mutant domain.[13] In summary, *otd* and *ems* are expressed in adjacent and slightly overlapping domains in the anterior embryonic fly brain. The function of these cephalic gap genes is required for the formation of specific regions of the anterior brain primordium.

Based on homology between homeobox sequences, orthologs of the *Drosophila otd* and *ems* genes have been isolated in various vertebrates including zebrafish, mouse and humans.[17,18] In mouse, the two vertebrate orthologs of the *otd* gene, *Otx1* and *Otx,2* are expressed in nested domains of the developing head and brain. *Otx1* transcripts first appear at approximately 8 days post coitum (dpc), whereas *Otx*2 expression is detectable earlier at the prestreak stage (5.5 dpc) within the entire epiblast and visceral endoderm prior to the onset of gastrulation. Subsequently, the domain of *Otx2* expression becomes restricted to the anterior region of the embryo, which includes a territory fated to give rise to forebrain and midbrain, defining a sharp boundary at the future midbrain-hindbrain boundary. *Otx1* expression is nested within this *Otx2* domain and subsequently becomes spatially and temporally restricted to the developing cortex and cerebellum. 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.[17,19] Analysis of *Otx1* mutants does not reveal any apparent defects in early brain development. However, later in development loss of *Otx1* function affects cortical neurogenesis and causes epilepsy. In addition, the development of eye and inner ear is impaired.[17,20] In contrast to *Otx1* mutant mice, *Otx2* null mice die early in embryogenesis and lack the rostral brain regions including forebrain, midbrain and rostral hindbrain due to defective anterior neuroectoderm specification.[17,21]

A comparison of the role of the *otd/Otx* genes in early brain patterning in *Drosophila* and mouse reveals striking similarities suggesting an evolutionary conservation of *otd/Otx* gene function. An interesting confirmation of the functional conservation in patterning the rostral brain can be carried out in cross-phylum rescue experiments. Ubiquitous overexpression of either human *Otx1* or human *Otx2* in an *otd* mutant fly embryo restores the anterior brain structures absent in the *otd* null mutant.[16] Similarly, overexpression of *Drosophila otd* in an *Otx1* null mouse embryo fully rescues epilepsy and corticogenesis abnormalities (but not inner ear defects).[17,22] Moreover, overexpression of a hybrid transcript consisting of the fly *otd* coding region fused to the 5' and

3' UTRs of *Otx2* restores the anterior brain patterning in *Otx2* null mutant mice including the normal positioning of the midbrain-hindbrain boundary.[23]

As is the case for the *otd/Otx* genes, two vertebrate orthologs of the *Drosophila ems* gene, *Emx1* and *Emx2*, have been identified. *Emx1* and *Emx2* expression in the mouse CNS is restricted to the forebrain, where largely overlapping expression patterns are seen. Whereas, *Emx1* expression only begins after neurulation, *Emx2* is already detectable around 8.5 dpc in the rostral neural plate.[19,24,25] Within the developing neocortex, *Emx2* is expressed in a high caudomedial to low rostrolateral gradient, which is contrasted by an opposed gradient of *Pax6* gene expression. Mutational inactivation of *Emx2* results in an expansion of the rostrolateral brain areas at the expense of the caudomedial neocortical areas. An opposite shift in regional identity is seen in the *Pax6* loss-of-function mutant. In the *Emx2* and *Pax6* double mutant, the cerebral cortex completely loses its identity and instead acquires characteristics of basal ganglia.[26,27] Whereas *Emx2* mutant mice die immediately after birth, *Emx1* mutant animals are postnatal viable and show rather subtle phenotypes that are restricted to the forebrain.[28,29] The regionalized expression patterns of the *ems/Emx* genes in the developing brain of *Drosophila* and mouse are remarkably similar, as is their ability to confer regional identity to the cells of a specific domain in the brain. Moreover, overexpression of a mouse *Emx2* transgene in an *ems* mutant background can rescue the brain phenotype of fly embryos.[15] Taken together, the similar spatiotemporal expression patterns and the high degree of functional equivalence between *Drosophila* and mouse suggest an evolutionarily conserved role of the *ems/Emx* and *otd/Otx* genes in anterior brain development.

The *Hox* Genes Pattern the Posterior Brain

The homeotic or *Hox* genes, encoding homeodomain transcription factors, were first discovered as crucial regulators of anteroposterior segment identity in the ectoderm of *Drosophila melanogaster*. Subsequently, *Hox* genes were found in a wide range of species where they have essential roles in many aspects of anteroposterior body axis patterning.[30,31] In *Drosophila*, the *Hox* genes are arranged along the chromosome in two gene clusters known as the *Antennapedia* (*ANT-C*) and *Bithorax* (*BX-C*) complexes. 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, there exists a correlation between the relative position of the genes within the cluster and their spatial and temporal expression pattern along the body axis; genes located towards the 3' end of the cluster are expressed more anteriorly and earlier in the embryo than are genes located towards the 5' end. This correlation has been termed spatial and temporal colinearity.[32] In mammals, *Hox* genes are arranged into four chromosomal clusters, termed *Hox A–D*, which contain between 9 and 11 *Hox* genes that can be assigned to 13 paralogous groups. Only the *Hox B* cluster comprises orthologs of all *Drosophila* homeotic genes. As in *Drosophila*, spatial and temporal colinearity is also observed among vertebrate *Hox* genes and more posterior acting genes impose their developmental specificities upon anterior acting genes.[32,33]

Hox gene expression in the developing CNS is a shared feature of a wide range of bilaterian animals, including protostomes such as insects or annelids and deuterostomes, such as hemichordates or vertebrates.[34-37] Remarkably, throughout the Bilateria, *Hox* gene orthologs are expressed in a similar anteroposterior order. In *Drosophila*, the expressions of *Hox* cluster genes delineate discrete domains in the embryonic brain and ventral nerve cord (Fig. 3A). Their anterior expression boundaries often coincide with morphologically defined neuromere compartment boundaries. Although the anteroposterior order of *Hox* gene expression domains largely follows the spatial colinearity rule known from ectodermal structures, one important difference is noteworthy: expression of the two 3'-most *Hox* genes of the *ANT-C* is inverted, in that the anterior expression boundary of *lab* lies posterior to that of *pb*.[34] Interestingly, this particularity of the *Hox* expression pattern in the CNS is common to fly and mouse. In vertebrates, *Hox* genes are expressed in the developing hindbrain and spinal cord. The relative anteroposterior order of *Hox* gene expression in the CNS of vertebrates is virtually identical to their arrangement in *Drosophila*, including the

inverted order of the *lab* and *pb* orthologs, *Hoxb-1* and *Hoxb-2* (Fig. 3B).[38] As more expression data from different protostome and deuterostome species becomes available, the ordered expression of *Hox* genes along the anteroposterior axis of the developing nervous system is likely to consolidate as a common feature of bilaterian animals.

In *Drosophila*, mutational inactivation of either of the homeotic genes *lab* or *Dfd* causes severe axonal patterning defects in the embryonic brain.[34] In *lab* null mutants, axonal projection defects are observed 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 projections in the tritocerebral commissure are absent or reduced. These brain defects are not due to deletions in the affected neuromere; neuronal progenitors are present and give rise to progeny in the mutant domain. However, these postmitotic progeny fail to acquire a neuronal identity, as indicated by the absence of neuronal markers and the lack of axonal and dendritic extensions (Fig. 3A). Comparable defects are seen in *Dfd* mutants in the corresponding mandibular/anterior maxillary domain, where the gene is expressed in the wild type brain.[34] Thus, the activity of the homeotic genes *lab* and *Dfd* is necessary to establish regionalized neuronal identity in the brain of *Drosophila*.

The mouse *lab* orthologs, *Hoxa-1* and *Hoxb-1*, are expressed in overlapping domains with a sharp anterior boundary coinciding with the presumptive rhombomere 3/4 border. Functional inactivation of *Hoxa-1* results in segmentation defects leading to a reduced size of rhombomeres 4 and 5 and defects in motor neuron axonal projections but the normal identity of rhombomere 4 is not altered.[39] In contrast, loss of *Hoxb-1* function has no influence on the size of rhombomere 4 but causes a partial transformation into a rhombomere 2 identity.[40] The *Hoxa-1, Hoxb-1* double 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 (Fig. 3B).[39] Thus, the concerted activity of *Hoxa-1* and *Hoxb-1* has a similar role in the specification of the regionalized neuronal identity as does their ortholog *lab* in the CNS of *Drosophila*. This suggests a functional conservation of *Hox* genes, in addition to a similar mode of expression, during nervous system development of bilaterian animals and supports the idea of a common origin of the CNS.

Evidence for a Tripartite Organization of the Brain

Comparative gene expression studies, as reviewed here for *Drosophila* and mouse, have been carried out in numerous protostome and deuterostome phyla.[36,41-44] The subdivision of the developing brain into an anterior region specified by genes of the *otd/Otx* family and a posterior region specified by genes of the *Hox* family appears to be a universal feature of bilaterian animals. In vertebrates and urochordates, a third embryonic domain along the anteroposterior neuraxis, characterized by overlapping expression of the *Pax2, Pax5* and *Pax8* genes, is located between the anterior *Otx* and the posterior *Hox* expressing regions of the embryonic brain.[45-47] In vertebrate brain development, this *Pax2/5/8* domain is located between the presumptive mesencephalon and metencephalon, where it plays a crucial role in development of the midbrain-hindbrain boundary (MHB) region or isthmus. Transplantation experiments, in which MHB tissue grafts are inserted to more rostral or caudal brain regions inducing ectopic mesencephalic-metencephalic structures, reveal an organizer function of the MHB. This organizer activity on the surrounding neural tissue is thought to be mediated by fibroblast growth factor 8 (Fgf8) and Wnt1 proteins, which are secreted by cells located in the MHB.[45,47] In early embryonic development of the vertebrate CNS, the homeobox gene *Gbx2* is expressed in the anterior hindbrain just posterior to the *Otx2* domain in the forebrain and midbrain. During gastrulation and early neurulation the MHB is established at the *Otx2/Gbx2* interface, where subsequently the expression domains of other MHB markers including *Pax2/5/8*, Fgf8, Wnt1 and *En1/2* are positioned (Fig. 4C). The two homeobox genes *Otx2* and *Gbx2* mutually repress one another and upregulation or downregulation of either gene shifts the position of the MHB accordingly.[45,47] Therefore, in vertebrates an antagonistic interaction between *Otx2* and *Gbx2* during early embryonic development is involved in the correct positioning of the MHB at their common interface.

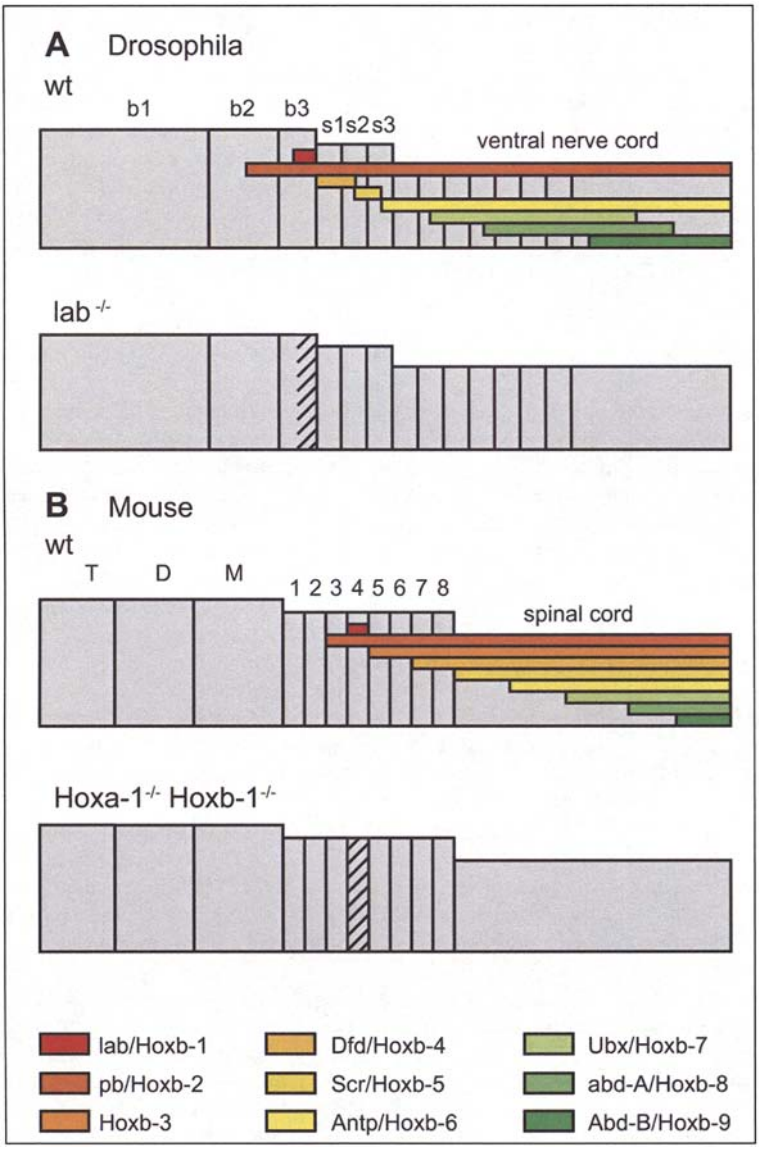

Figure 3. Simplified schematic comparison of *Hox* gene expression domains and mutant phenotypes in the CNS of *Drosophila* and mouse. Schematic representations of the embryonic brain with anterior towards the left and posterior towards the right. A) Expression domains of the homeotic genes of the Antennapedia and Bithorax complexes in the CNS of *Drosophila* (see text for gene nomenclature). 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 (dashed lines). B) Expression of the *Hox* genes *Hoxb-1* to *Hoxb-9* in the developing mouse CNS. *Hoxa-1*$^{-/-}$ and *Hoxb-1*$^{-/-}$ double mutant embryos (*Hoxa-1*$^{-/-}$; *Hoxb-1*$^{-/-}$) lose rhombomere 4 identity (dashed lines). Abbreviations: T, telencephalon; D, diencephalon; M, mesencephalon; 1-8, rhombomeres 1-8; (for other abbreviations see Fig. 2). Modified and reprinted with permission from: Hirth F et al. Development 1998; 125: 1579-1589. © The Company of Biologists Limited.

Gene expression studies indicate that a similar tripartite ground plan for anteroposterior regionalization of the embryonic brain is also present in *Drosophila*. The *Drosophila* genome contains two genes, *Pox neuro* (*Poxn*) and *Pax2*, which are together considered to be orthologs of the *Pax2/5/8* genes.[48] Remarkably, expression of both orthologs is present at the interface of *otd* and the *Drosophila Gbx2* ortholog *unplugged* (*unpg*), anterior to a *Hox*-expressing region (Fig. 4A,B).[44] Although *Poxn* and *Pax2* are expressed in a segmentally reiterated pattern along the entire embryonic CNS, their expression at the *otd/unpg* interface is exceptional in two ways. The two genes are expressed in adjacent domains delineating together a transversal stripe of the brain and this is the only position along the neuraxis where expression of both genes coincides with a brain neuromere boundary, the deutocerebral-tritocerebral boundary (DTB) (Fig. 4A,B).[44] Analyses of either *otd* or *unpg* mutants reveal a mutually repressive function of the two genes during early brain patterning. Thus, in *otd* mutant embryos a rostral extension of the *unpg* expression domain is observed (in addition to the deletion of the anterior brain). On the other hand, mutational inactivation of the *unpg* gene results in a caudal shift of the posterior limit of *otd* expression.[44] Therefore, in both *Drosophila* and mouse, the early interaction of *otd/Otx2* and *unpg/Gbx2* is essential for the correct positioning of an intermediate brain domain characterized by a sharply delimited *otd/Otx2* and *unpg/Gbx2* interface and the expression of *Pax2/5/8* genes. In contrast to vertebrates, mutational inactivation of the *Drosophila Pax2/5/8* orthologs *Poxn* or *Pax2* does not appear to result in brain patterning defects. Moreover, to date, there is no evidence of an organizer activity at the fly DTB, suggesting that the organizer function at the *otd/Otx2* and *unpg/Gbx2*

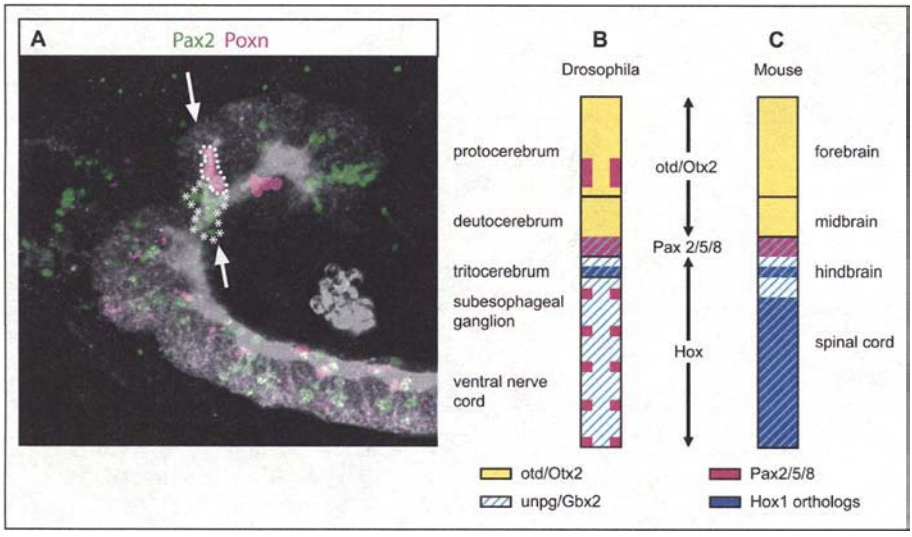

Figure 4. Tripartite organization of the embryonic CNS in *Drosophila* and mouse. A) Expression of *Pax2* and *Poxn* in the brain of stage 13/14 embryos. At the deutocerebral-tritocerebral boundary (indicated by white arrows), *Pax2* (white dots) and *Poxn* (white asterisks) are expressed in adjacent domains forming a transversal line in the CNS (immunolabelled with antiHRP and shown in grey). B,C) The expression of *otd/Otx2, unpg/Gbx2, Pax2/5/8* and *Hox1* gene orthologs in the developing CNS of *Drosophila* (B) and mouse (C). (In this schematic, anterior is towards the top and posterior is towards the bottom.) In both cases, *otd/Otx2* is expressed in the anterior nervous system rostral to a *Hox*-expressing region in the posterior nervous system. In addition, a *Pax2/5/8*-expressing domain positioned at the interface between the anterior *otd/Otx2* domain and the posteriorly abutting *unpg/Gbx2* expression domain is common to both nervous systems. Modified and reprinted with permission from: Hirth F et al. Development 2003; 130: 2365-2373. © The Company of Biologists Limited.

interface might have emerged after the protostome/deuterostome divergence that separated insects and vertebrates. In fact, an organizer activity of the MHB region has so far only been demonstrated for vertebrate species within deuterostomes.

In summary, current comparative data indicates that similar genetic patterning mechanisms act in anteroposterior regionalization of the developing brain in *Drosophila* and vertebrate species and establish a common, evolutionarily conserved tripartite ground plan. This suggests that a corresponding tripartite organization of the developing brain was already present in the last common bilateral ancestor of insects and vertebrates.

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