An urbilaterian origin of the tripartite brain: developmental genetic insights from Drosophila

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SUMMARY

Studies on expression and function of key developmental control genes suggest that the embryonic vertebrate brain has a tripartite ground plan that consists of a forebrain/midbrain, a hindbrain and an intervening midbrain/hindbrain boundary region, which are characterized by the specific expression of the Otx, Hox and Pax2/5/8 genes, respectively. We show that the embryonic brain of the fruitfly Drosophila melanogaster expresses all three sets of homologous genes in a similar tripartite pattern. Thus, a Pax2/5/8 expression domain is located at the interface of brain-specific otd/Otx2 and unpg/Gbx2 expression domains anterior to Hox expression regions. We identify this territory as the deutocerebral/tritocerebral boundary region in the embryonic Drosophila brain. Mutational inactivation of otd/Otx2 and unpg/Gbx2 result in the loss or misplacement of the brain-specific expression domains of Pax2/5/8 and Hox genes. In addition, otd/Otx2 and unpg/Gbx2 appear to negatively regulate each other at the interface of their brain-specific expression domains. Our studies demonstrate that the deutocerebral/tritocerebral boundary region in the embryonic Drosophila brain displays developmental genetic features similar to those observed for the midbrain/hindbrain boundary region in vertebrate brain development. This suggests that a tripartite organization of the embryonic brain was already established in the last common urbilaterian ancestor of protostomes and deuterostomes.

Key words: Brain development, Brain evolution, Midbrain/hindbrain boundary, Otx, Hox, Pax, Drosophila melanogaster

INTRODUCTION

Classical phylogenetic, neuroanatomical and embryological studies have led to the view of an independent evolutionary origin of protostome and deuterostome brains. Accordingly, bilaterians have been divided into two major groups with different CNS morphologies: the gastroneuralia, which are characterized by a ventral nerve cord, include protostomes such as arthropods, annelids and molluscs; and the notoneuralia, which are characterized by a dorsal nerve cord, include all chordates (Siewing, 1985; Brusca and Brusca, 1990; Nielsen, 2001). By contrast, more recent studies examining the expression patterns and functions of orthologous genes in embryonic nervous systems have revealed similarities in the embryonic brains of protostomes, such as annelids and arthropods, and deuterostomes like tunicates and mammals. These results suggest that lophotrochozoan and ecdysozoan invertebrate brains and chordate brains of vertebrates, acanulates and urochordates are all characterized by a rostral region specified by genes of the otd (oc – FlyBase)/Otx family and a caudal region specified by genes of the Hox family (reviewed by Bruce and Shankland, 1998; Arendt and Nübler-Jung, 1999; Hirth and Reichert, 1999; Holland and Holland, 1999; Reichert and Simeone, 1999; Wada and Satoh, 2001; Reichert, 2002).

In ascidian and vertebrate chordates, a Pax2/5/8 expression domain is located between the anterior Otx and the posterior Hox expression regions of the embryonic brain (reviewed by Holland and Holland, 1999; Wada and Satoh, 2001). In vertebrate brain development, this Pax2/5/8 expression domain is positioned at the interface of the brain-specific Otx2 and Gbx2 expression domains; it is an early marker for the isthmic organizer at the midbrain-hindbrain boundary (MHB), which controls the development of the midbrain and the anterior hindbrain (reviewed by Liu and Joyner, 2001; Rhinn and Brand, 2001; Wurst and Bally-Cuif, 2001). The central role of this MHB region in brain development together with the conserved expression patterns of Pax2/5/8 genes in this region have led to the proposal that a fundamental characteristic of the ancestral chordate brain was its tripartite organization characterized by Otx, Pax2/5/8 and Hox gene expressing regions (Wada et al., 1998). To investigate whether protostomes also possess a tripartite organization of the brain, and to gain insight into the evolution of the bilaterian brain, we carried out a comparative analysis of expression and function in the ecdysozoan...
Chiang et al., 1995, MATERIALS AND METHODS

Our study reveals striking similarities in the expression and function of genes that pattern the embryonic brains of Drosophila and vertebrates. We find that a Pax2/5/8 expressing domain is located between an anterior otd-expressing region and a posterior Hox-expressing region in the embryonic brain of Drosophila. Moreover, in Drosophila, as in vertebrates, we find that this Pax2/5/8 expressing domain is positioned at the interface between the otd/Otx2 expression domain and a posteriorly abutting unplugged/Gbx2 expression domain. Finally, we demonstrate that inactivation of otd/Otx2 or of unplugged/Gbx2 results in comparable effects on mispositioning or loss of brain-specific expression domains of orthologous genes in both embryonic brain types. These developmental genetic similarities indicate that the tripartite organization already existed in the brain of the last common urbilateral ancestor of insects and chordates.

RESULTS

Pax2 and Poxn expression in the embryonic brain

In the embryonic CNS of vertebrates, the Pax2, Pax5 and Pax8 genes are expressed in specific domains that overlap in the presumptive MHB region (reviewed by Liu and Joyner, 2001; Rhinn and Brand, 2001; Wurst and Bally-Cuif, 2001). Drosophila has two Pax2/5/8 orthologues, Pax neuro (Poxn) and Pax2 (Noll, 1993; Fu and Noll, 1997).

The embryonic brain of Drosophila can be subdivided into the protocerebrum (PC or b1), deutocerebrum (DC or b2) and tritocerebrum (TC or b3) of the supra-oesophageal ganglion and the mandibular (S1), maxillary (S2) and labial (S3) neuromeres of the sub-oesophageal ganglion. Expression of engrafted (en) delimits these subdivisions by marking their most posterior neurones (reviewed by Hartmann and Reichert, 1998) (Fig. 1). Because of morphogenetic processes, such as the beginning of head involution, the neuraxis of the embryonic brain curves dorso-posteriorly within the embryo. Accordingly, henceforth anteroposterior coordinates refer to the neuraxis rather than the embryonic body axis.

We first determined whether Pax2 is expressed in specific domains of the Drosophila brain, by analysing its expression pattern using in situ hybridization, immunolabelling and lacZ reporter gene expression. Pax2 transcripts initially appear during gastrulation and at stage 9/10 are observed in a segmentally reiterated pattern of the developing procephalic and ventral neuroectoderm, with its anteriormost expression domain located at the future deutocerebral-tritocerebral boundary (Fig. 2A, arrow). Expression of Pax2 transcripts in the developing brain begins at stage 10/11 and is most prominent in a longitudinal stripe at the medial part of the protocerebrum and in a transversal stripe at the posterior border of the deutocerebrum (Fig. 2B-D, arrowheads). Immunolabelling with a Pax2-specific polyclonal antibody (Fu and Noll, 1997) revealed that Pax2 protein distribution resembles that of Pax2 transcripts, as does a Pax2-lacZ reporter gene expressing β-galactosidase (Fig. 3A-C). In addition to its expression in the developing anterior brain, Pax2 expression is also seen in six to eight cells located at the lateral margin of each hemisegment throughout the more posterior CNS regions of the sub-oesophageal ganglion and ventral nerve cord (Fig. 3C).

To determine the expression of the second Drosophila Pax2/5/8 orthologue, we characterized Poxn expression using immunolabelling and lacZ reporter genes. Poxn protein is first detected in the developing brain at the end of germ band extension (stage 10/11) in two stripes of the procephalic neuroectoderm, which subsequently become restricted to the posterior protocerebrum and the posterior deutocerebrum (Fig. 3D,E). Poxn expression in more posterior regions of the CNS also occurs in segmentally reiterated patterns (Fig. 3F) (Bopp

LASER CONFOCAL MICROSCOPY

For laser confocal microscopy, a Leica TCS SP microscope was used. Optical sections ranged from 0.4 to 2 μm, recorded in line average mode with picture size of 512x512 pixels. Captured images from optical sections were arranged and processed by the use of IMARIS (Bitplane). Figures were arranged and labelled by the use of Adobe Photoshop.
A comparison between Pax2 and Poxn expression domains reveals, that Pax2 and Poxn are never co-expressed in the same cells of the CNS. Moreover, expression of Pax2 and Poxn does not occur at a comparable anteroposterior position along the neuraxis (Fig. 3I), with one exception. This exception is in the posterior deutocerebrum where adjacent Pax2 and Poxn expression domains define a transversal domain immediately anterior to the tritocerebral brain neuromere (arrows in Fig. 3G,H). This transversal domain of adjacent Pax2 and Poxn expression is distinguishable from 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 (compare Fig. 3H with Fig. 1D). We refer to this transversal domain of adjacent Pax2/5/8 orthologue expression as the deutocerebral-tritocerebral boundary (DTB) region.

It is important to note that the DTB is located anterior to the expression domain of the Drosophila Hox1 orthologue labial (lab), which is expressed in the posterior tritocerebrum (Hirth et al., 1998). Moreover, the DTB is located posterior to the expression domain of the Drosophila Otx orthologue otd in the protocerebrum and anterior deutocerebrum (Hirth et al., 1995). Thus, in Drosophila as in vertebrates, a Pax2/Poxn (Pax2/5/8) expression domain is located between the anterior otd/Otx2 and the posterior Hox-expressing regions. This raises the question of whether the DTB in the embryonic Drosophila brain might have developmental genetic features similar to those observed for the MHB in vertebrate brain development.

Gene expression patterns at the deutocerebral-tritocerebral boundary region

In the embryonic vertebrate brain, Otx2 is expressed anterior to and abutting Gbx2. The future MHB as well as the overlapping domains of Pax2, Pax5 and Pax8 expression are positioned at this Otx2-Gbx2 interface (Liu and Joyner, 2001; Rhinn and Brand, 2001; Wurst and Bally-Cuif, 2001). To investigate if comparable expression patterns are found in the embryonic fly brain, we determined the brain-specific expression of the Drosophila Gbx2 orthologue unplugged.

Fig. 1. Expression of engrailed (en) delimits the neuromeric subdivisions of the embryonic brain and ventral nerve cord. Laser confocal microscopy of stage 13/14 wild-type embryos, reconstructions of optical sections. (A,C) Frontal views; (B,D) lateral views. The bracket in B indicates the extend of optical sections used to reconstruct the frontal views of A,C. (A,B) Immunolabelling with a neurone-specific anti-HRP antibody (red). (C,D) Double-immunolabelling with anti-HRP (red) and anti-EN (green, yellow). The embryonic brain can be subdivided into the protocerebrum (PC or b1), deutocerebrum (DC or b2) and tritocerebrum (TC or b3) of the supraesophageal ganglion and the mandibular (S1), maxillary (S2) and labial (S3) neuromeres of the sub-oesophageal ganglion. en-expressing cells delimit the posterior boundaries of the PC (en-b1), DC (en-b2) and TC (en-b3), and of the mandibular (en-S1), maxillary (en-S2) and labial (en-S3) neuromeres.

Fig. 2. Whole-mount in situ hybridization of Pax2 transcripts in wild-type embryos. (A,B,B’) Lateral views, (C,D,D’) dorsal views; anterior is towards the left. (A) By stage 9/10, Pax2 expression is detectable in a segmentally reiterated pattern of the developing procephalic and ventral neuroectoderm, with its anteriormost expression domain located at the future deutocerebral-tritocerebral boundary (arrow). (B-D) At stage 14, Pax2 transcripts are most prominent in a longitudinal stripe at the medial part of the protocerebrum (arrows) and in a transversal stripe (arrowheads) at the posterior border of the deutocerebrum. Asterisks in C,D indicate Pax2 expression in cells associated with the developing hypopharyngeal organ outside the CNS (see also Fig. 3A). (B’) Simplified summary scheme of B with brain-specific Pax2 expression domains (grey) shown in relation to protocerebral and deutocerebral neuromere boundaries (unbroken lines). (D’) Simplified summary scheme of D with brain-specific Pax2 expression domains (grey) shown in relation to protocerebral and deutocerebral neuromere boundaries (unbroken lines).
(unpg) in relation to that of otd, using immunolabelling and an unpg-lacZ reporter gene that expresses β-galactosidase like endogenous unpg (Chiang et al., 1995). The otd gene is expressed in the protocerebrum and anterior deutocerebrum of the embryonic brain (Fig. 4A,B) (Hirth et al., 1995), as well as in midline cells in more posterior regions of the CNS (Fig. 4C) (Finkelstein et al., 1990; Wieschaus et al., 1992). Expression of unpg-lacZ in the embryonic CNS is first detected at stage 8 in neuroectodermal and mesectodermal cells at the ventral midline, with an anterior limit of expression at the cephalic furrow (Chiang et al., 1995). Subsequently, the unpg expression domains in the CNS widen and have their most anterior border in the posterior deutocerebrum (Fig. 4D-F). Double immunolabelling of OTD and β-galactosidase revealed that the posterior border of the brain-specific otd expression domain coincides with the anteriormost border of the unpg expression domains along the anteroposterior neuraxis (Fig. 4G,H, arrows). There is no overlap of otd and unpg expression in the brain or in more posterior regions of the CNS (Fig. 4G-I).

These findings indicate that the otd-unpg interface is positioned at the anterior border of the DTB. This was confirmed by additional immunolabelling studies examining unpg-lacZ, otd, Poxn and en expression in the protocerebral/deutocerebral region of the embryonic brain. Thus, double immunolabelling of OTD and EN confirms that the posterior border of otd expression extends beyond the protocerebral en-b1 stripe into the anterior deutocerebral domain (Fig. 5A). Labelling OTD and POXN confirms that the posterior border of otd expression extends beyond the pro-otic domain (Fig. 5B). Labelling EN and β-galactosidase, which is indicative of unpg expression, confirms that the anteriormost unpg expression domain overlaps with the poxn expression domain of the DTB (Fig. 5D). Therefore, in terms of overall gene expression patterns, we find that a transversal domain of adjacent Pax2/5/8 orthologue expression is located anterior to the deutocerebral/tritocerebral neuromere boundary. Note that expression of Pax2 and Poxn are never observed in the same cells. The apparent co-expression of Pax2 and Poxn shown in G (white) is an artefact caused by superimposed optical sections. Asterisks in A indicate Pax2 expression in cells associated with the developing hypopharyngeal organ outside the CNS.

Fig. 3. Pox neuro (Poxn) and Pax2 are expressed in distinct domains in the embryonic brain and ventral nerve cord. Laser confocal microscopy of stage 13/14 embryos, reconstructions of optical sections. (A-L) P{lacZ}Pax2 Δ122/+. (A,D,G) Frontal views, (B,E,H) lateral views, (C,F,I) ventral views. The bracket in B indicates the extent of optical sections used to reconstruct the frontal views of A,D,G, the dotted line demarcates the deutocerebral/tritocerebral neuromere boundary. (A-C) Double-immunolabelling with anti-HRP (red) and anti-β-gal (green, yellow). (D-F) Double-immunolabelling with anti-HRP (red) and anti-POXN (blue). (G-I) Triple-immunolabelling with anti-HRP (red), anti-β-gal (green, yellow) and anti-POXN (blue, white); arrows in G,H indicate Pax2 and Poxn expression at the same anteroposterior position along the neuraxis in the posterior deutocerebrum where they define a transversal domain. This transversal domain of adjacent Pax2/5/8 orthologue expression is located anterior to the deutocerebral/tritocerebral neuromere boundary. Note that expression of Pax2 and Poxn are never observed in the same cells. The apparent co-expression of Pax2 and Poxn shown in G (white) is an artefact caused by superimposed optical sections. Asterisks in A indicate Pax2 expression in cells associated with the developing hypopharyngeal organ outside the CNS.
Mutation of *otd* and of *unpg* result in loss or misplacement of brain-specific gene expression domains

In mammalian brain development, homozygous *Otx2-null* mutant embryos lack the rostral brain, including the MHB-specific *Pax2/5/8* expression domain (Acampora et al., 1995; Matsuo et al., 1995; Ang et al., 1996), whereas *Gbx2* null mutants misexpress *Otx2* and *Hoxb1* in the brain (Wassarman et al., 1997). Moreover, *Otx2* and *Gbx2* negatively regulate each other at the interface of their expression domains (Li and Joyner, 2001; Liu and Joyner, 2001; Martinez-Barbera et al., 2001; Rhinn and Brand, 2001; Wurst and Bally-Cuif, 2001; Ye et al., 2001). To test if similar regulatory interactions occur in the embryonic brain of *Drosophila*, we analysed the expression of the corresponding orthologues in *otd* and *unpg* mutant embryos.

**Fig. 4.** *otd* and *unpg* expression domains in the embryonic brain and ventral nerve cord. Laser confocal microscopy of stage 13/14 embryos, reconstructions of optical sections. (A-C) Wild type, (D-I) *P{lacZ}unpg*Δ54+. (A,D,G) Frontal views, (B,E,H) lateral views, (C,F,I) dorsal views. The brackets in B,E,H indicate the extend of optical sections used to reconstruct the frontal views of A,D,G, respectively. (A-C) Double-immunolabeling with anti-HRP (red) and anti-OTD (green, yellow). (D-F) Double-immunolabeling with anti-HRP (red) and anti-β-gal (green, yellow). (G-I) Double-immunolabeling with anti-OTD (red) and anti-β-gal (green, yellow); arrows indicate interface of *otd* and *unpg* expression at the anterior border of the deutocerebral-tritocerebral boundary (DTB) region. Note that a direct interface of the *otd* and *unpg* expression domains only occurs on the ventral (according to neuraxis) side of the brain; on the opposing side, a small gap between the two expression domains is seen (H, arrow).

**Fig. 5.** Brain-specific expression of *otd, unpg, en* and *Poxn*. Laser confocal microscopy of stage 13/14 embryos, reconstructions of optical sections, frontal views. The extend of optical sections used to reconstruct the frontal views is more dorsal (according to neuraxis) than for Fig. 4A,D,G. (A,B) Wild type, (C,D) *P{lacZ}unpg*Δ54+. (A) Double-immunolabeling with anti-OTD (red) and anti-EN (green, yellow). (B) Double-immunolabeling with anti-OTD (red) and anti-POXN (green, yellow). (C) Double-immunolabeling with anti-β-gal (red) and anti-EN (green, yellow). (D) Double-immunolabeling with anti-β-gal (red) and anti-POXN (green, yellow). Arrowheads indicate en-b1 stripes, arrows indicate en-b2 stripes. Asterisks in A-C indicate non-neuronal expression domains of OTD and EN in the foregut.
In otd-null mutant embryos, the protocerebrum is absent because protocerebral neuroblasts are not specified (Hirth et al., 1995; Younossi-Hartenstein et al., 1997). Analysis of unpg, en and Poxn expression in otd-null mutant embryos revealed that the anteriormost border of unpg expression shifts anteriorly into the anterior deutocerebrum (Fig. 6A,B), while Poxn fails to be expressed in the deutocerebrum (Fig. 6C). In contrast to

**Fig. 6.** Altered expression patterns of unpg, en and Poxn in brains of otd–/– embryos. Laser confocal microscopy of stage 13/14 embryos, reconstructions of optical sections. (A) Frontal view, (B,C) lateral views. (A,B) otdΔ101; P[3’lacZ]unpg37/+; (C) otdΔ101. The extend of optical sections used to reconstruct the frontal view in A is the same as in Fig. 3B. Broken rectangles in the summary schemes indicate the region of the embryonic brain shown at higher magnification in B,C. (A,B) Triple-immunolabelling with anti-HRP (red), anti-β-gal (green, yellow) and anti-EN (blue, white). Arrows indicate the anterior shift of brain-specific unpg expression into the anterior deutocerebrum (compare with Fig. 4D,E). Note that the protocerebrum is missing in otd mutants (Hirth et al., 1995), resulting in the absence of en-b1-expressing cells, whereas en-b2-expressing cells delimiting the deuto-tritocerebral boundary are present (A,B, arrowheads). (C) Double-immunolabelling with anti-HRP (red) and anti-POXN (green, yellow). Arrow indicates the normal position of the posterior POXN stripe in the posterior deutocerebrum, which is absent in the otd-null mutant embryo. PC, protocerebrum; DC, deutocerebrum; TC, tritocerebrum; Sub, sub-oesophageal ganglion; VNC, ventral nerve cord.

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**Fig. 7.** Altered expression patterns of otd and lab in brains of unpg−/− embryos. Laser confocal microscopy of stage 13/14 embryos, reconstructions of optical sections. (A,C,D) Frontal views, (B,E,F) lateral views. (A-D,F) P[3’lacZ]unpg37; (E) P[3’lacZ]unpg37/+; (F) unpg−/+. The extend of optical sections used to reconstruct the frontal views in A,C,D is the same as in Fig. 3B; this results in increased visualization of gut-specific otd and en expression. Broken rectangles in the summary schemes indicate the region of the embryonic brain shown as magnified view in B,F. (A,B) Double-immunolabelling with anti-OTD (red) and anti-β-gal (green, yellow). Arrows indicate the posterior shift of brain-specific otd expression into the anterior-most unpg expression domain. (C) Double-immunolabelling with anti-OTD (red) and anti-EN (green, yellow). Arrows indicate the posterior shift of brain-specific otd expression up to the deutocerebral en-b2 stripe into the posterior deutocerebrum (compare with Fig. 5A). Broken line indicates non-neuronal expression domains of OTD and EN in the foregut. (D) Double-immunolabelling with anti-OTD (red) and anti-POXN (green, yellow). Arrows indicate that the posterior border of brain-specific otd expression extends posteriorly up to the Poxn expression domain of the DTB (compare with Fig. 5B). Broken line indicates non-neuronal expression domains of OTD and POXN in the foregut. (E,F) Double-immunolabelling with anti-LAB (red) and anti-β-gal (green, yellow) in unpg−/− versus unpg+/− embryos; the broken line demarcates the tritocerebral/mandibular neuromere boundary. Arrowheads indicate the anterior shift of lab expression from the posterior tritocerebrum (E) (Hirth et al., 1998) into the anterior tritocerebrum (F). PC, protocerebrum; DC, deutocerebrum; TC, tritocerebrum; Sub, sub-oesophageal ganglion; VNC, ventral nerve cord.
Invertebrate brain development, the Pax2 gene, and subsequently the Pax5 and Pax8 genes, are among the first genes expressed at the Otx2/Gbx2 interface, followed by the overlapping expression of En1 and Fgf8 genes (Ye et al., 2001). Inactivation of Pax2, Pax5, En1 or Fgf8 results in the loss of the midbrain and cerebellum because of a failure to maintain development of this brain region (reviewed by Joyner, 1996; Wassef and Joyner, 1997; Acampora and Simeone, 1999; Martinez et al., 1999; Liu and Joyner, 2001; Rhinn and Brand, 2001; Wurst and Bally-Cuif, 2001). In Drosophila, no obvious brain phenotypes were seen after mutational inactivation of Pax2, Pax5, En1 and Fgf8 genes (Liu and Joyner, 2001; Rhinn and Brand, 2001; Wurst and Bally-Cuif, 2001).

**DISCUSSION**

Comparative developmental studies in urochordates and vertebrates have led to the notion that the basic ground plan for the chordate brain consists of a forebrain/midbrain region characterized by Otx gene expression, a hindbrain region characterized by Hox gene expression, and an intervening boundary region characterized by expression of Pax2, Pax5, En1 and Fgf8 genes (Wada et al., 1998; Holland and Holland, 1999; Wada and Satoh, 2001). Our data show that this tripartite ground plan is also found in the insect brain (Fig. 8). This suggests that a corresponding, evolutionarily conserved, tripartite organization also characterized the brain of the last common ancestor of insects and chordates.

A comparison of the brain-specific topology of gene expression patterns that define this tripartite organization in *Drosophila* and in mouse suggests that the vertebrate midbrain/hindbrain boundary (MHB) region corresponds to the insect deutocerebral-tritocerebral boundary (DTB) region. However, this is not always the case, one might expect that other patterning genes that characterize the MHB region are also expressed at the insect DTB. Although this expectation is fulfilled for the segment-polarity genes *en* and *wingless* (*wg*) in *Drosophila*, these two genes are expressed at the borders of all CNS neuromeres, as well as at parasegmental boundaries in the...
epidermis; hence, their expression may not be indicative of brain-specific requirements.

In addition to remarkable similarities in orthologous gene expression between insects and chordates, our study also shows that several functional interactions among key developmental control genes involved in establishing the Pax2/5/8-expressing MHB region of the vertebrate brain are also conserved in insects. Thus, in the embryonic brains of both fly and mouse, the intermediate boundary regions, DTB and MHB, are positioned at the interface of otd/Otx2 and unpg/Gbx2 expression domains (Fig. 8A,B). These boundary regions are deleted in otd/Otx2-null mutants and mispositioned in unpg/Gbx2-null mutants. Moreover, otd/Otx2 and unpg/Gbx2 genes engage in cross-regulatory interactions, and appear to act as mutual repressors at the interface of their brain-specific expression domains. However, not all of the functional interactions among genes involved in MHB formation in the mouse appear to be conserved at the Drosophila DTB. Thus, in the embryonic Drosophila brain, no patterning defects are observed in null mutants of Pax2, Poxn, en or bnl. It remains to be seen if these genes play a role in the postembryonic development of the Drosophila brain.

It is conceivable that the similarities of orthologous gene expression patterns and functional interactions in brain development evolved independently in insects and vertebrates. However, a more reasonable explanation is that an evolutionary conserved genetic program underlies brain development in all bilaterians. This would imply that the generation of structural diversity in the embryonic brain is based on positional information that has been invented only once during evolution and is provided by genes such as otd/Otx2, unpg/Gbx2, Pax2/5/8 and Hox, conferring on all bilaterians a common plan that characterizes the developing chordate brain. This implies that a corresponding tripartite organization already existed in the brain of the last common urbilaterian ancestor of insects and chordates. Therefore, we propose an urbilaterian origin of the brain of the last common urbilaterian ancestor of insects and chordates. Therefore, we propose an urbilaterian origin of the brain of the last common urbilaterian ancestor of insects and chordates. This implies that a corresponding tripartite organization already existed in the brain of the last common urbilaterian ancestor of insects and chordates. Therefore, we propose an urbilaterian origin of the brain of the last common urbilaterian ancestor of insects and chordates.

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