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Segment polarity and DV patterning gene expression reveals segmental organization of the *Drosophila* brain

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SUMMARY

The insect brain is traditionally subdivided into the trito-, deuto- and protocerebrum. However, both the neuromeric status and the course of the borders between these regions are unclear. The *Drosophila* embryonic brain develops from the procephalic neurogenic region of the ectoderm, which gives rise to a bilaterally symmetrical array of about 100 neuronal precursor cells, called neuroblasts. Based on a detailed description of the spatiotemporal development of the entire population of embryonic brain neuroblasts, we carried out a comprehensive analysis of the expression of segment polarity genes (*engrailed, wingless, hedgehog, gooseberry distal, mirror*) and DV patterning genes (*muscle segment homeobox, intermediate neuroblast defective, ventral nervous system defective*) in the procephalic neuroectoderm and the neuroblast layer (until stage 11, when all neuroblasts are formed). The data provide new insight into the segmental organization of the procephalic neuroectodem and evolving brain. The expression patterns allow the drawing of clear demarcations between trito-, deuto- and protocerebrum at the level of identified neuroblasts. Furthermore, we provide evidence indicating that the protocerebrum (most anterior part of the brain) is composed of two neuromeres that belong to the ocular and labral segment, respectively. These protocerebral neuromeres are much more derived compared with the trito- and deutocerebrum. The labral neuromere is confined to the posterior segmental compartment. Finally, similarities in the expression of DV patterning genes between the *Drosophila* and vertebrate brains are discussed.

Key words: CNS, Brain development, Neuroblasts, Segment polarity genes, Dorsoventral patterning genes, Segmentation, *Drosophila*

INTRODUCTION

In order to integrate multiple sensory input and generate appropriate behavioural responses, the central nervous system (CNS) has to be composed of region-specific structures that fulfil particular functions. The formation of these structures can be correlated back to the activity of patterning genes during early embryonic development. Molecular and genetic tools as well as manipulation techniques make *Drosophila* a suitable model system for the investigation of developmental processes that underlie patterning and cellular diversity in the CNS. So far, investigations on CNS development in the *Drosophila* embryo have mainly focused on its less complex truncal region – the ventral nerve cord. The ventral nerve cord arises from multipotent stem cells, called neuroblasts (NBs), which delaminate from the ventral neurogenic region in a segmentally repeated pattern (Doe, 1992; Hartenstein and Campos-Ortega, 1984). Segmental patterning defines functional units, which can then be refined during further development. For example, within a segment (neuromere) each NB acquires a unique identity and produces a specific cell lineage (for a review, see Doe and Technau, 1993). Genetic mechanisms of neuromere and NB formation in the ventral nerve cord are quite well understood (for a review, see Campos-Ortega, 1993), and some advances in understanding the processes that lead to the specification of individual NB identities have recently been made. Specific identities appear to be conferred to presumptive NBs in the neuroectoderm by positional cues. For example, the segment polarity genes subdivide the neuroectoderm into transverse rows along the anteroposterior axis and the dorsoventral patterning genes in longitudinal columns along the DV axis. The superimposition of the expression patterns of both gene groups establishes a Cartesian coordinate system of positional cues in which the fate of a particular NB depends on the respective ‘quadrant’ in which it is formed (for reviews, see Bhat, 1999; Skeath, 1999).

The situation is much more complex in the procephalic neuroectoderm and the brain. The insect brain develops highly organized neuropil structures, such as the mushroom bodies and the central complex (e.g. Bullock and Horridge, 1965; Hanesch et al., 1989; Hanström, 1928; Strausfeld, 1976), that are required for behavioural functions such as olfactory learning and memory or the control of locomotor activity (e.g. Heisenberg, 1998; Strauss and Heisenberg, 1993); these structures have no equivalents in other ganglia. The key towards elucidating the origin of these structures lies in an understanding of the segmental organization of the brain. However, the segmental pattern in the head is highly derived

and its metameric organization has been intensely debated (e.g. Boyan and Williams, 2000; Haas et al., 2001; Hirth et al., 1995; Jürgens et al., 1986; Rempel, 1975; Rogers and Kaufman, 1996; Schmidt-Ott et al., 1994). In *Drosophila* the expression of *engrailed* and *wingless* argues for the existence of four pregnathal segments: the intercalary, antennal, ocular and labral segments (Schmidt-Ott et al., 1994; Schmidt-Ott et al., 1995; Schmidt-Ott and Technau, 1992). Although it has been suggested that each head segment contributes to the brain (Schmidt-Ott and Technau, 1992), the arrangement and boundaries of the corresponding neuromeres, and the origin and identities of their progenitor cells are largely unknown.

Based on a detailed description of the entire population of brain NBs and their spatiotemporal pattern of segregation from the neuroectoderm (Urbach et al., 2003), we have investigated the expression of segment polarity genes and dorsoventral patterning genes in the procephalic neuroectoderm, as well as in the individually identified brain NBs through to stage 11, when the full complement of NBs has formed. The work provides new insight into the positional cues expressed in the procephalic neuroectoderm and the segmental organization of the evolving brain. The data strongly support the view that the pregnathal *Drosophila* head is composed of four segments, and we now attribute to each of the four pregnathal segments a corresponding neuromere. Furthermore, we provide evidence that the protocerebrum consists of two neuromeres, which derive from the ocular and labral segment. The segmental character of these neuromeres is less conserved compared with the trito- and deutocerebrum, deriving from the intercalary and antennal segment. Finally, we discuss similarities in the expression of dorsoventral patterning genes between the *Drosophila* and vertebrate brain.

MATERIALS AND METHODS

Drosophila strains

The following fly strains were used: Oregon R (wild type), *engrailed-lacZ* (ryXho25) (Hama et al., 1990), *hedgehog-lacZ* (16E) (Mohler et al., 1995) (kindly provided by J. Mohler), *mirror-lacZ* (Broadus et al., 1995; McNeill et al., 1997) (kindly provided by H. McNeill and M. Simon), *muscle segment homeobox-lacZ* (rH96) (Isshiki et al., 1997) (kindly provided by A. Nose), *ventral nervous system defective-lacZ* (kindly provided by F. Jimenez) and *wingless-lacZ* (Broadus et al., 1995).

Staging, flat preparation and mounting of embryos

Staging of the embryos was carried out according to Campos-Ortega and Hartenstein (Campos-Ortega and Hartenstein, 1997); additionally, we used the trunk NB pattern (Doe, 1992) as a further morphological marker for staging. Flat preparations of the head ectoderm of stained embryos and mounting were carried out as described previously (Urbach et al., 2003).

Antibodies and immunohistochemistry

Embryos were dechorionated, fixed and immunostained according to previously published protocols (Patel, 1994). The following primary antibodies were used: rabbit-anti-Asense (1:5000) (Brand et al., 1993) (kindly provided by Y.-N. Yan), rabbit-anti-Deadpan (1:300) (Bier et al., 1992) (kindly provided by H. Vaessin), mouse-anti-β-Galactosidase (1:500, Promega), rabbit-anti-β-Galactosidase (1:2500, Cappel), rat-anti-Gooseberry-distal (16F12 and 10E10, 1:2) (Zhang et al., 1994) (kindly provided by B. Holmgren), mouse-anti-Invected (4D9,1:4) (Patel et al., 1989) (Developmental Studies Hybridoma Bank), mouse-anti-Ladybird early (1:2) (Jagla et al., 1997) (kindly provided by K. Jagla), rabbit-anti-Muscle segment homeobox (1:500; kindly provided by M. P. Scott), rabbit-anti-Ventral nervous system defective (1:2000) (McDonald et al., 1998) (kindly provided by F. Jimenez) and mouse-anti-Wingless (1:10, Developmental Studies Hybridoma Bank), anti-DIG-AP (1:1000, Roche). The secondary antibodies (Dianova) were either biotinylated (goat anti-mouse, goat anti-rabbit) or alkaline phosphatase-conjugated (goat anti-mouse, goat anti-rabbit, goat anti-rat) and diluted 1:500.

Whole-mount in situ hybridization

DIG-labelled *intermediate neuroblast defective* (*ind*) RNA probe (kindly provided by M. P. Scott) was synthesized with T7 polymerase and *Hin*dIII linearized pNB40-ind as a template according to the manufacturers protocol (Roche). The hybridization on embryos was performed as described previously (Plickert et al., 1997; Tautz and Pfeifle, 1989).

Documentation

Embryos were viewed under a Zeiss Axioplan equipped with Nomarski optics using 40×, 63× and 100× oil immersion objectives. Pictures were digitized with a CCD camera (Contron progress 3012) and different focal planes were combined using Adobe Photoshop 6.0. Semi-schematic presentations are based on camera lucida drawings.

RESULTS

The metameric expression of segment polarity genes is conserved in the early brain

In the trunk neuroectoderm, segment-polarity genes are expressed in stereotypical segmental stripes, and in NBs that delaminate from these domains, subdividing each neuromere along the AP axis (Bhat, 1996; Broadus et al., 1995; Chu-LaGraff and Doe, 1993; Skeath et al., 1995; Zhang et al., 1994). In the pregnathal head region the expression domains of segment polarity genes are less obvious, but previous analysis of *engrailed* and *wingless* expression in the head peripheral ectoderm, and of PNS mutant phenotypes, support the existence of four pregnathal segments in *Drosophila*: the intercalary, antennal, ocular and labral segments (Schmidt-Ott et al., 1994; Schmidt-Ott et al., 1995; Schmidt-Ott and Technau, 1992). However, the identity and organization of brain structures deriving from these segments is still obscure. In order to obtain evidence concerning the number and extent of the brain neuromeres, and to map the position of their boundaries, we analysed the expression of segment polarity genes, including *wingless*, *hedgehog*, *gooseberry-distal*, *engrailed*, *invected* and *mirror*. The spatiotemporal pattern of their expression was traced in the neuroectoderm and in the NB-layer until stage 11, when all brain NBs are formed. The data (detailed in Figs 1, 2 and 4) show that segmental expression is retained for most of the investigated segment polarity genes in both the developing head ectoderm (procephalon) and brain NBs, providing landmarks for the definition of segmental domains within the developing brain NB pattern.

engrailed (*en*) expression domains in the trunk define the posterior segmental compartments (DiNardo et al., 1985; Poole et al., 1985), from which NBs of row 6 and 7 and NB1-2 derive (Broadus et al., 1995). In the pregnathal head (neuroectoderm: Fig. 1B,C,E,F; Fig. 2A,C,E,J,K,L) (NBs: Fig. 1A,D; Fig.

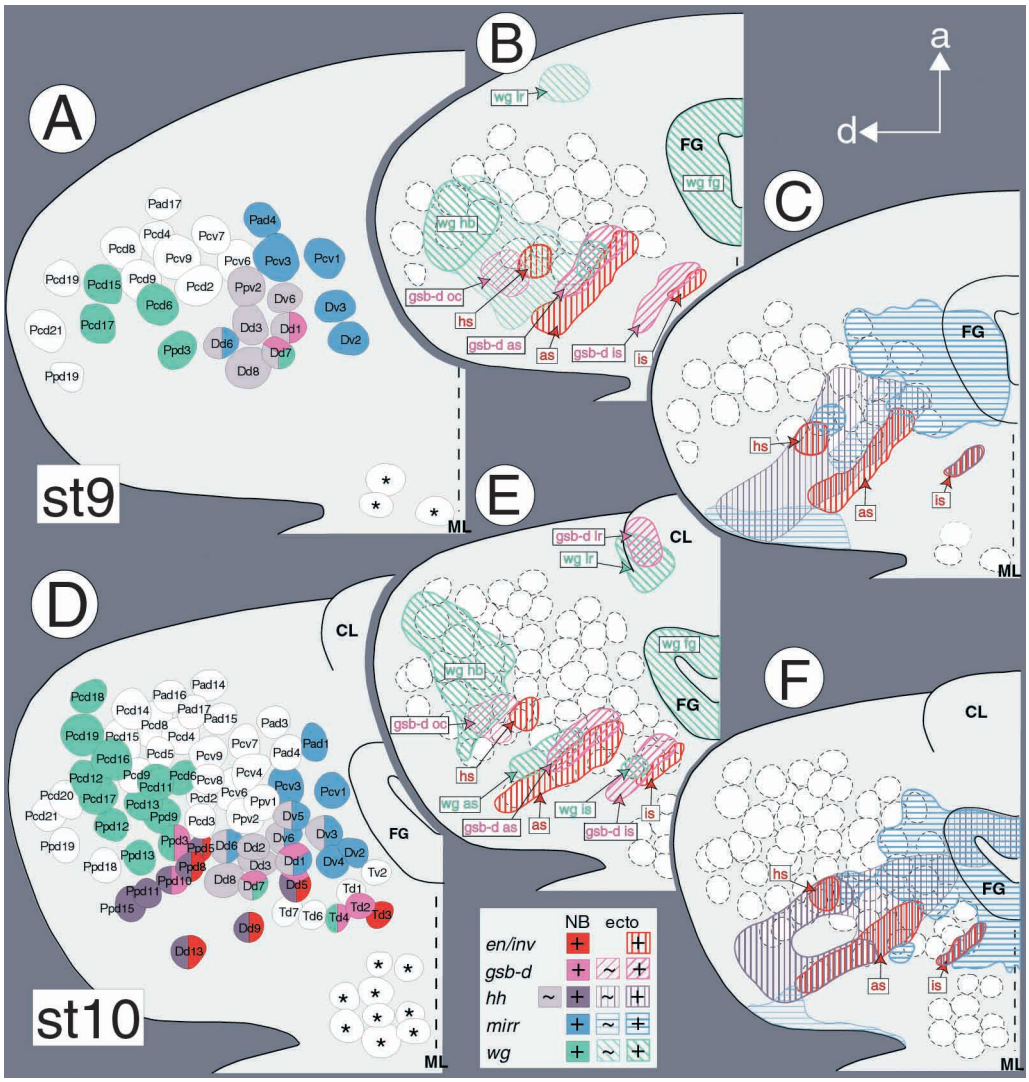


Fig. 1. Expression of segment polarity genes at embryonic stages 9 (st9) and 10 (st10). Colour code indicates the expression pattern of *engrailed-lacZ*/Invected protein (*en/inv*; red), *hedgehog-lacZ* (*hh*; purple), *mirror-lacZ* (*mirr*; blue), *wingless-lacZ*/Wingless protein (*wg*; green), and Gooseberry-distal protein (*gsb-d*; pink) at the level of identified brain NBs (A,D) and procephalic neuroectoderm (B,C,E,F). All panels show semi-schematic representations of a ventral view of the left half of head flat preparations; anterior (a) is towards the top and dorsal (d) is towards the left. Nomenclature of brain NBs is according to their position in the trito- (T), deuto- (D) and protocerebrum (P) [for details of the nomenclature see Urbach et al. (Urbach et al., 2003)]. In (B,C,E,F), thick hatching marks strong expression and thin hatching marks weak expression of the respective gene; for comparison, NBs underlying the peripheral ectoderm are encircled by broken lines. The expression of *en*, *gsb-d* and *wg* is confined to intermediate and dorsal brain NBs (A,D) and corresponding sites of the peripheral procephalic ectoderm (B,C,E,F). By stage 10, Gsb-d is detected in the ectoderm of all four pregnathal head segments (E). In the *gsb-d* ocular domain (*gsb-d* oc) and two corresponding NBs (Ppd3, Ppd10) *gsb-d* expression is transient (compare with Fig. 2). It is also transiently expressed (between stages 10 and 11) in the clypeolabral ectoderm [in the *gsb-d* labral spot, which is partly colocalized with the *wg* labral spot (E)] from which no NBs emerge (see also Fig. 2). *mirr-lacZ* is not segmentally expressed in the procephalic neuroectoderm; it is observed in the invaginating foregut and flanking neuroectoderm, and is additionally detected in a more intermediate ectodermal antennal spot (from which Dd6 arises). A large *wg* domain extends from the antennal into the ocular head region (B), but later separates into an antennal stripe (*wg* as) and ocular head blob (*wg* hb) (E). *hh-lacZ* expression accumulates in posterior regions of the antennal and ocular ectoderm (F; see also Fig. 2J). (A,D) Note that a large number of identified brain NBs (especially protocerebral) do not express any segment polarity gene. For a detailed description, see text. Stars indicate mandibular NBs. as, en antennal stripe; CL, clypeolabrum; FG, foregut; gsb-d as, gsb-d antennal stripe; gsb-d is, gsb-d intercalary stripe; gsb-d lr, gsb-d labral spot; gsb-d oc, gsb-d ocular domain; hs, en head spot; is, en intercalary stripe; ML, ventral midline; wg as, wg antennal stripe; wg lr, wg labral spot; wg fg, wg expression in the foregut; wg is, wg intercalary spot.; wg hb, wg ocular head blob [for nomenclature of *en* and *wg* expression domains in the procephalic ectoderm, see Schmidt-Ott and Technau (Schmidt-Ott and Technau, 1992)].

2B,D,F,I,M) we find *en* expression as follows: from late stage 8 in the posterior ectoderm of the antennal segment (*en* antennal stripe; en as) from which four deutocerebral NBs (Dv8, Dd5, Dd9, Dd13) delaminate; from stage 9 in a small ectodermal domain in the posterior part of the ocular segment, the *en* head spot (en hs), from which two protocerebral NBs (Ppd5, Ppd8) evolve; and from stage 10 in an ectodermal stripe in the posterior intercalary segment (*en* intercalary stripe; en is), which gives rise to three to four tritocerebral NBs (Tv4, Tv5, Td3, Td5). Furthermore, from stage 11 onwards, En is weakly detected in the anteriormost ectoderm of the procephalon corresponding to the region of the 'anterior dorsal hemispheres' (*en* dh) (Rogers and Kaufman, 1996; Schmidt-Ott and Technau, 1992) (Fig. 2I-N) (see also Urbach et al., 2003). We identified about 10 weakly En-positive NBs, which delaminate from the *en* dh (Fig. 2I,M). Thus, consistent with earlier results (Schmidt-Ott and Technau, 1992), we find that all four pregnathal head segments contribute to the early embryonic brain. The spatial distribution of the En-positive NBs closely corresponds to the *en* domains of their origin in the ectoderm. This suggests they demarcate the posterior borders of the respective brain neuromeres (Fig. 4).

In the trunk, *hedgehog* (*hh*) matches *en* expression (Mohler and Vani, 1992; Tabata et al., 1992). This is also the case for the intercalary segment in the pregnathal head ectoderm (Fig. 1C,F;

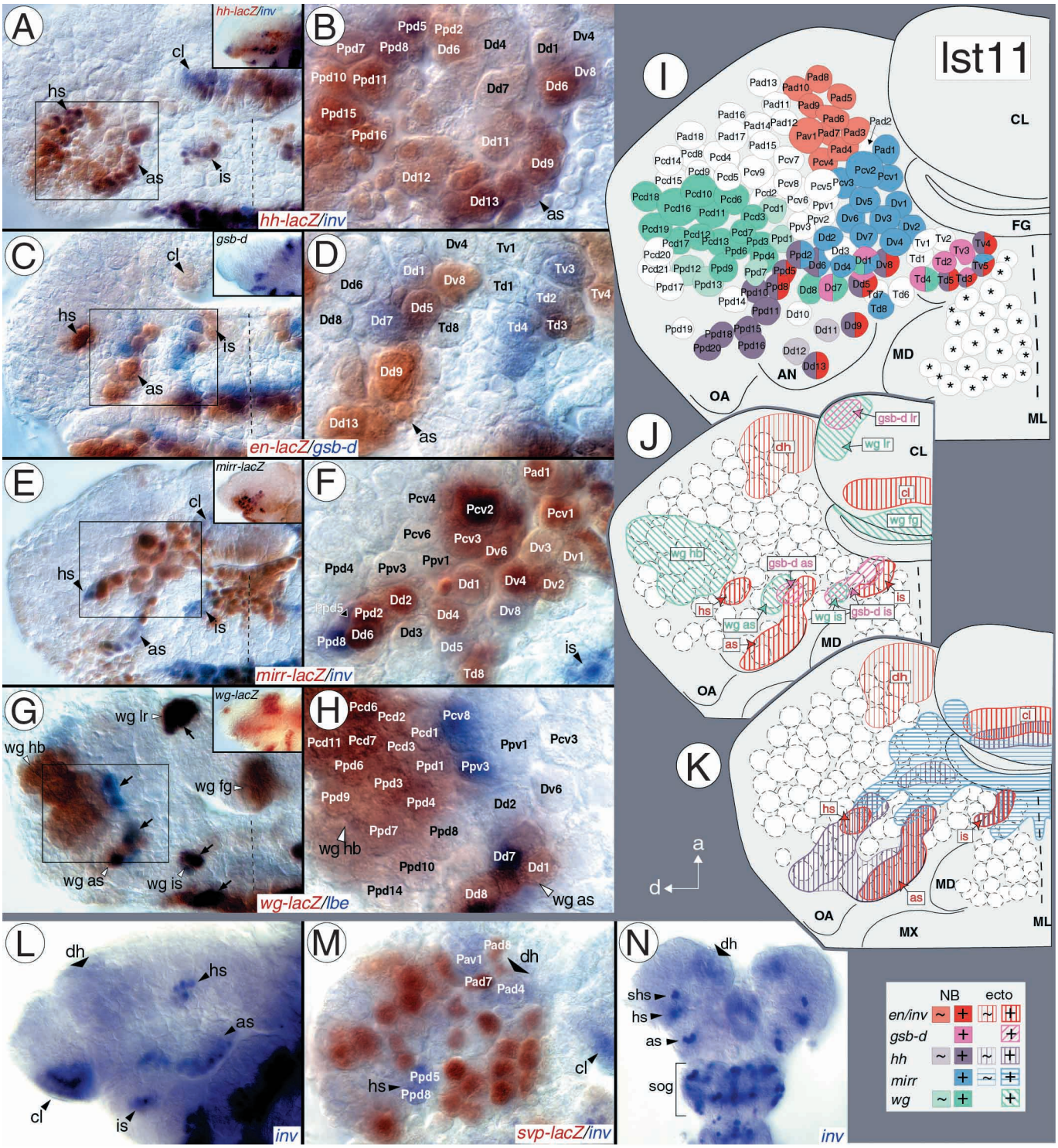


Fig. 2K). By contrast, the En-positive antennal stripe and head spot are only subfractions of the large *hh-lacZ* domain, which, between stage 9 and 10, encompasses the antennal segment and the posterior part of the ocular segment. We find that all NBs delaminating from this domain express *hh-lacZ* (Fig. 1A,D). From stage 10 onwards, *en* expressing NBs maintain a strong *hh-lacZ* signal, whereas *hh-lacZ* subsequently diminishes in the neuroectoderm and in NBs between the en antennal stripe and head spot (compare Fig. 1D,F with Fig. 2I,J). Additionally, *hh-lacZ*-expressing NBs positioned dorsally to the *en/hh-lacZ*-co-expressing Ppd5 and Ppd8 (both NBs demarcating part of the posterior border of the ocular neuromere), appear to prolong the boundary between the deuto- and protocerebrum in the dorsal direction (Fig. 1D, Fig. 2I, Fig. 4).

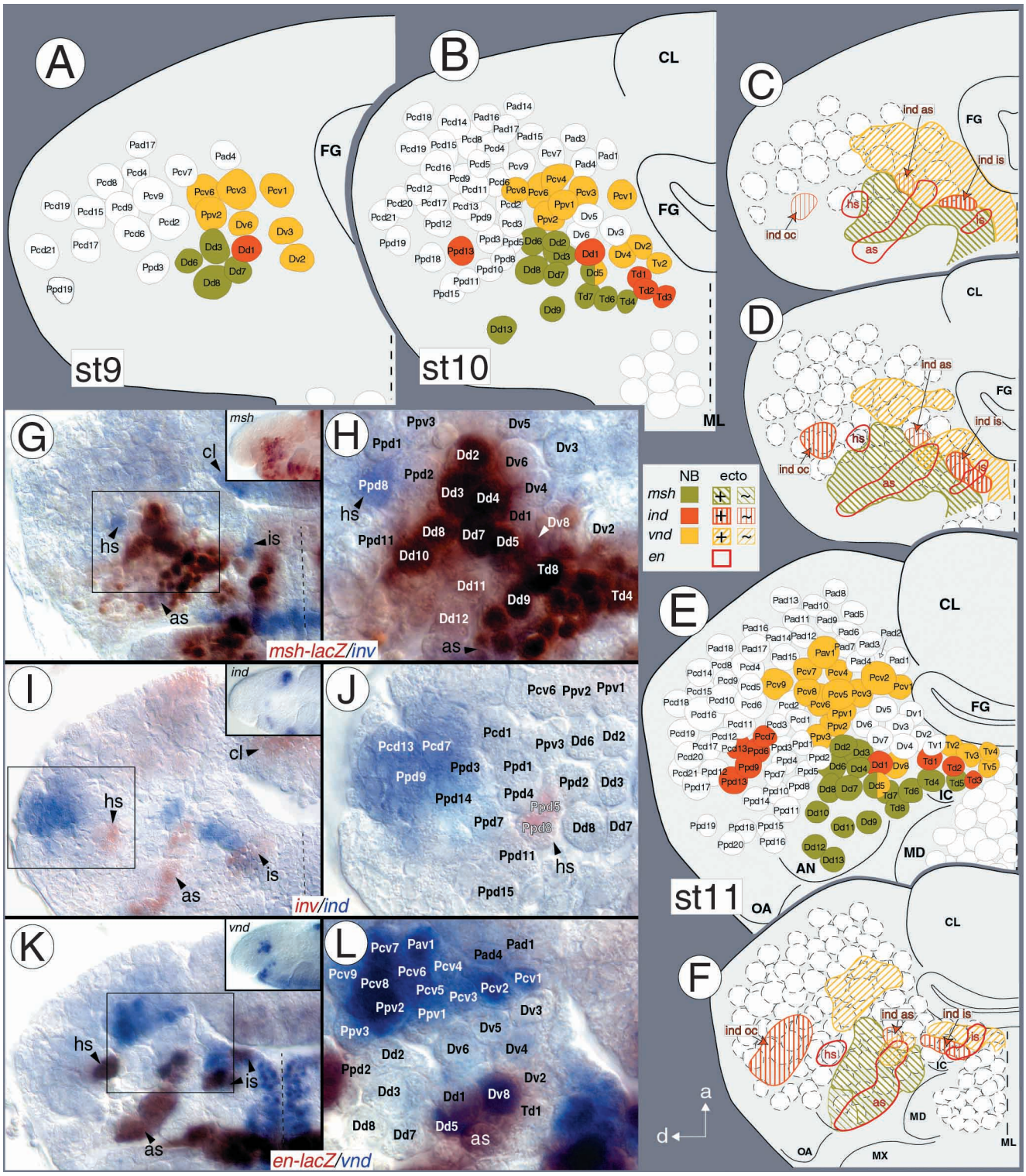
From late stage 8 onwards, Wingless (Wg) protein is expressed in a neuroectodermal domain spanning a broad area

Fig. 2. Expression of segment polarity genes at embryonic stage 11 (st11). (A,C,E,G) Left half of head flat preparations double labelled for segment polarity gene expression and *en-lacZ*, *invected* (*inv*) or *ladybird* (*lbe*); focus is on the peripheral head ectoderm; broken line marks the ventral midline; insets depict lateral views of stage 11 whole-mount preparations. (B,D,F,H) Close-ups of regions indicated in A,C,E,G by black frames; focus is on the level of NBs; immunopositive NBs are indicated by white inscription, immunonegative NBs are indicated by black inscription. (A,B) Only part of the antennal/ocular *hh-lacZ* domain co-expresses *inv*. Note the strong *hh-lacZ* signal in the dorsal Ppd5, Ppd8, Ppd10, Ppd11, Ppd15 and Ppd16. (C) *gsb-d* is downregulated in the ocular neuroectoderm, and is detectable in four tritocerebral NBs deriving from the *gsb-d* intercalary stripe, and in three deutocerebral NBs deriving from the *gsb-d* antennal stripe (D,I,J). (E,F) *mirr-lacZ* expression shows no segmental pattern and is mainly limited to the ventral part of the PNR and corresponding NBs. (G) *lbe* is segmentally expressed in the procephalic neuroectoderm [arrows; for details, see Urbach and Technau (Urbach and Technau, 2003)], where it is co-expressed with *wg*, except in the *wg* hb and *wg* fg. (H) *lbe* is co-expressed with *wg* in Dd7 but not in the ocular Ppv3 and Pcv8. (I) Segment polarity gene expression in identified brain NBs at stage 11; nomenclature of brain NB has been described previously (Urbach et al., 2003). Colour intensity reflects weak (~) and strong (+) expression levels of *inv*, *hh-lacZ* and *wg*. Stars indicate mandibular NBs. (J,K) Segment polarity gene expression in the peripheral procephalic ectoderm. (L-N) *inv* expression in the dorsal hemispheres (dh). (L) Lateral view of late stage 11 head, showing a faint *inv* expression in the dh. Note that *en* expression in the dh is not detected using *en-lacZ* and was only observed with anti-Inv antibodies from late stage 11 onwards. (M) Left half of a head flat preparation. The dh comprises about 10 Inv-positive NBs (as depicted in I). (N) *inv* expression in the brain and sub-oesophageal ganglion (SOG) of a stage 16 embryo (horizontal view). Note, that Inv staining in the dh corresponds to the pars intercerebralis of postembryonic stages. The secondary head spot (shs) marks a small group of cells which secondarily separates from the hs (see Schmidt-Ott and Technau, 1992). AN, IC, MD, MX, antennal, intercalary, mandibular and maxillary segment, respectively; CL, clypeolabrum; FG, foregut; ML, ventral midline; OA, Bolwig organ/optic lobe anlagen; as, *en* antennal stripe; cl, *en* expression in the clypeolabrum; dh, *en* expression in the dorsal hemispheres; hs, *en* head spot; is, *en* intercalary stripe; shs, *en* secondary head spot; gsb-d as, *gsb-d* antennal stripe; gsb-d is, *gsb-d* intercalary stripe; gsb-d lr, *gsb-d* labral spot; wg as, *wg* antennal stripe; wg fg, *wg* expression in the foregut; wg hb, *wg* ocular head blob; wg is, *wg* intercalary spot; wg lr, *wg* labral spot.

of the ocular and the anterior antennal segment (and in the invaginating foregut) (see also Baker, 1988; van den Heuvel et al., 1989). This becomes clearer in En/Wg double labelling at stage 9, revealing that the en hs is localized within this Wg domain (Fig. 1B). In contrast to earlier observations (Richter et al., 1998; Younossi-Hartenstein et al., 1996), we find that, at that stage, Wg is already detectable in about 4-5 protocerebral NBs (Pcd6, Pcd15, Pcd7, Ppd3; Fig. 1A), derived from the region with strongest Wg expression [which later corresponds to the *wg* head blob; for nomenclature of *wg* expression domains in the procephalic ectoderm, see Schmidt-Ott and Technau (Schmidt-Ott and Technau, 1992)]. Furthermore, Wg is faintly expressed in the deutocerebral Dd7 (Fig. 1A) emerging from the antennal part of the Wg domain (Fig. 1B), which corresponds to the later *wg* antennal stripe (Fig. 1E, Fig. 2G,J). By stage 10, when the *wg* head blob is clearly distinguishable from the *wg* antennal stripe (Fig. 1E), about 10-12 Wg-positive NBs have emerged from this domain (Fig. 1D). In addition, we found a small, spot-like *wg* domain in the intercalary segment (Fig. 1E; *wg* intercalary spot) from which a single NB (Td4) delaminates (Fig. 1D). Thus, all three *wg* domains, the intercalary, antennal and ocular (head blob), contribute to the anlage of the brain. From late stage 9 an additional *wg* domain is visible in the ectodermal anlage of the clypeolabrum (Fig. 1B,E, Fig. 2G,J), which is the *wg* counterpart to the En/Inv-positive region in the 'dorsal hemispheres' [wg labral spot in Schmidt-Ott and Technau (Schmidt-Ott and Technau, 1992)]. Upon double labelling for either *asense* or *deadpan* (both are general markers for neural precursor cells) and *wg*, in embryos between stage 9 and 11 we could not identify any NB emerging from the *wg* labral spot. By stage 11 the number of *wg* expressing NBs originating from the ocular head blob has increased to about 16-20 (Fig. 2H,I), which is more than 25% of the total number of identified protocerebral NBs. Three Wg-positive NBs are identified in the deutocerebrum and one in the tritocerebrum (Fig. 2I).

The *gooseberry* (*gsb*) locus encodes two closely related proteins, Gsb-distal (Gsb-d) and Gsb-proximal (Baumgartner et al., 1987; Bopp et al., 1986), which are both expressed in the developing ventral nerve cord (Gutjahr et al., 1993; Ouellette et al., 1992). Gsb-d is segmentally expressed at high levels in all row 5 and 6 NBs, as well as in a median row 7 NB (NB 7-1) (Broadus et al., 1995; Zhang et al., 1994). We analysed the expression of *gsb-d* during early neurogenesis in the head region, and found segmental expression of Gsb-d to be conserved in parts of the pregnathal head ectoderm and deriving NBs (for details see Fig. 1A,B,D,E; Fig. 2C,D,I,J). Gsb-d/En double labelling show that the *gsb-d* intercalary and antennal stripes are expressed anteriorly to the corresponding *en* stripes, and are partly overlapping with the *en* stripes (Fig. 1B,E, Fig. 2C,J). Consequently, NBs from the posterior part of the *gsb-d* stripe in the tritocerebrum and deutocerebrum co-express *en* (Td3, Dd5; Fig. 1D, Fig. 2D,I), and those from the anterior part co-express *wg* (Td4, Dd1 and Dd7; as seen in Gsb-d/Wg double labelling; Fig. 1A,D, Fig. 2I, and data not shown), resembling the situation in the ventral nerve cord. However, Dd8 and all Wg-positive protocerebral NBs do not co-express Gsb-d (except for Ppd3 which, like Ppd10, transiently expresses *gsb-d* during stage 10; Fig. 1D). Gsb-d can also be detected at a low level in ganglion mother cells of the respective NBs, but fades away in NBs and their progeny during germ band retraction. Expression of the protein in the brain is completely downregulated at stage 13 (data not shown).

In the trunk, *mirror* (*mirr*)-*lacZ* is expressed in segmental ectodermal stripes giving rise to *mirr-lacZ*-positive NBs of row 2 and several NBs that flank row 2 at stage 11 (Broadus et al., 1995; McNeill et al., 1997). The pattern of *mirr-lacZ* expression in the procephalic neuroectoderm and brain NBs differs significantly from the trunk. We find no evidence of a segmental arrangement of *mirr-lacZ* expression in the procephalon (for details, see Fig. 1A,C,D,F, Fig. 2E,F,I,K). Interestingly, regarding the DV axis, *mirr-lacZ* is mainly limited to the ventral part of the pNR and corresponding NBs (as confirmed by *mirr-lacZ*/Vnd double staining, although there is a faint dorsal *mirr-lacZ* expression, in the region of the later invaginating optic lobe anlage; Fig. 1A,C,D,F, Fig. 2I,K), and is, at stage 9/10, roughly complementary to *en*, *wg* and *gsb-d* expression, the domains of which are mainly confined to



intermediate and dorsal regions of the pNR (Fig. 1B,C,E,F). At stage 11, expression extends towards the dorsal part of the antennal neuroectoderm (Fig. 2E,K) and is observed in all NBs of the ventral deutocerebrum, as well as in two tritocerebral (Tv5, Td8) and four ventral, protocerebral NBs (Pad1, Pcv1, Pcv2, Pcv3; Fig. 2F,I). Although expression is also found in the clypeolabrum (Fig. 2E,K), we did not identify *mirr-lacZ*-positive labral NBs.

Expression of dorsoventral patterning genes during early brain development

In addition to the segment polarity genes, the dorsoventral patterning genes *ventral nervous system defective* (*vnd*), *intermediate neuroblast defective* (*ind*) and *muscle segment homeobox* (*msh*) have been shown to confer positional information to the truncal neuroectoderm, which also contributes to the specification of NBs (reviewed by Skeath,

Fig. 3. Expression of DV patterning genes at embryonic stages 9 (st9),10 (st10) and 11 (st11). Colour code indicates the expression of *msh* (*msh-lacZ* and Msh protein), *ind* transcripts and *vnd* (*vnd-lacZ* and Vnd protein) in identified NBs (A,B,E) and the procephalic neuroectoderm (C,D,F). *en* expression domains are shown for comparison. Orientation of the semi-schematic representations is as in previous figures. (A,C) Stage 9; note the spot-like *ind* expression in the intercalary (ind is; *ind* intercalary spot), antennal (ind as; *ind* antennal spot) and ocular (ind oc; *ind* ocular spot) head region; the *ind* antennal spot, in contrast to the intercalary, overlaps completely with the ventral *vnd* domain; the emerging Dd1 does not express *vnd*. (B,D) Stage 10; *vnd* is already downregulated in part of the ventral antennal ectoderm and corresponding Dv3 and Dv6, and is also not expressed in the newly developed Dv5. *msh* and *vnd* expression overlaps in a small area of the antennal ectoderm and emerging Dd5. (E,F) Stage 11; note that most of the identified brain NBs (especially protocerebral NBs) do not express any DV patterning gene. (G,I,K) Left half of head flat preparations double labelled for *en* expression (*en-lacZ*; Inv protein) and *msh* (*msh-lacZ*, Msh protein), *ind* (transcripts) or *vnd* (*vnd-lacZ* and Vnd protein), respectively; broken line marks the ventral midline; insets depict lateral views of stage 11 whole-mount heads. (H,J,L) Close-ups of regions indicated in G,I,K by frames (focus on the level of NBs). Immunopositive NBs are indicated by white, immunonegative NBs by black inscription. (G,H) *msh-lacZ*/Inv-antibody double labelling. The anterior border of the *msh* expression domain is positioned immediately posterior to the *en* hs (G), and runs between the deutocerebral and ocular protocerebral NBs (H). (I,J) DIG *ind* mRNA/Inv antibody double labelling. (K,L) *en-lacZ*/Vnd antibody double labelling. Vnd protein has disappeared in most parts of the antennal ectoderm (K) and NBs (L); note the dorsally directed extension of *vnd* domains in the antennal and ocular procephalic regions. The antennal *vnd* expression overlaps dorsally with the En-positive Dv8 and Dd5. The posterior border of the ocular *vnd* domain runs between deuto- and protocerebral NBs (L). AN, IC, MD, MX, antennal, intercalary, mandibular and maxillary segment, respectively; CL, clypeolabrum; FG, foregut; ML, ventral midline; OA, Bolwig organ/optic lobe anlagen; as, en antennal stripe; cl, *en* expression in the clypeolabrum; hs, *en* head spot; is, *en* intercalary stripe; ind is, *ind* intercalary spot; ind as, *ind* antennal spot; ind oc, *ind* ocular spot.

1999). For the head and brain, a detailed analysis of the expression of these genes has not yet been undertaken. In order to elucidate their putative role in patterning the head and brain, we analysed the expression of *vnd*, *ind* and *msh* in the procephalic ectoderm and NBs in the early embryo (until stage 11). Although our data are consistent with their role in dorsoventral patterning being principally conserved in the procephalon, we also find significant differences in their patterns of expression compared with the trunk (as outlined in the following and in Figs 3, 4).

At the blastodermal stage, Ventral nervous system defective protein (Vnd) is expressed in bilateral longitudinal stripes corresponding to the most ventral neuroectodermal column (Jimenez et al., 1995; McDonald et al., 1998; Mellerick and Nirenberg, 1995), and is by stage 11 detected in all ventral and two intermediate NBs of the ventral nerve cord. Interestingly, the latter co-express *en* and are located in the posterior compartment of each truncal neuromere (Chu et al., 1998; McDonald et al., 1998; Shao et al., 2002; Uhler et al., 2002). At gastrulation the ventral longitudinal *vnd* domain reaches anteriorly across the cephalic furrow into the procephalic neuroectoderm (data not shown). By stage 9, *vnd* maps in the ventral neuroectoderm of the prospective intercalary, antennal and ocular segment (which is confirmed by En/Vnd double labelling; Fig. 3C) and is observed in ventral NBs of the antennal (Dv2, Dv3, Dv6) and ocular neuromere (Pcv1, Pcv3, Pcv6, Ppv2) (Fig. 3A). It appears as if the dorsal part of the Vnd-positive antennal neuroectoderm partly co-expresses *ind* at that stage (Fig. 3C), but the NB Dd1, which emerges from this ectodermal region expresses only *ind* and not *vnd* (Fig. 3A). This is possibly due to the transient expression of *vnd* in most parts of both the ventral antennal ectoderm (compare Fig. 3C with 3D) and corresponding NBs: by stage 10 Vnd is detected in the ventral Dv2, Dv4 and Dd5, but is already downregulated in Dv3 and Dv6, and by stage 11 it is confined to Dd5 and the new Dv8 (Fig. 3B,E,L). As a consequence of the downregulation of *vnd* (compare Fig. 3C,D,F,K), some ventral deutocerebral NBs, which delaminate between stage 9 and 11 from this domain were not observed to express *vnd* (e.g. Dv1, Dv5, Dv7; Fig. 3B,D,E,L). By stage 11 Vnd is seen in four tritocerebral (Tv2, Tv3, Tv4, Tv5), in two deutocerebral (Dd5, Dv8), and in a cluster of about 13 protocerebral NBs (Fig. 3E,L). Interestingly, *vnd* expression expands along the posterior border of the *en* intercalary stripe (en is), and is also significantly extended dorsally into the *en* antennal stripe (Fig. 3D,F,K) and the NBs delaminating from there. The fact that *vnd* and *en* are co-expressed in Tv5 and in Dd5, Dv8 (Fig. 3L, Fig. 4) is in agreement with findings in the ventral nerve cord, where these genes are co-expressed in two intermediate NBs (Chu et al., 1998; Shao et al., 2002). This indicates that *vnd* demarcates the ventral part of the posterior border in trunk as well as in brain neuromeres. Furthermore, the posterior border of the ocular *vnd* domain (including the NBs Pcv1, Pcv2, Pcv3, Ppv1, Ppv2, Ppv3) abutts dorsally the En-positive NBs Ppd5 and Ppd8 (deriving from the *en* head spot; Fig. 1D, Fig. 3E,K,L), supporting the view that these NBs demarcate the posterior border of the ocular neuromere (Fig. 4).

intermediate neuroblast defective (*ind*) is expressed in the blastoderm in a bilateral longitudinal column (intermediate column neuroectoderm) just dorsal to the *vnd* domains. In the trunk, at stage 9 (when *ind* mRNA is no longer present in the neuroectoderm), it is expressed in all intermediate NBs and finally, at stage 11, it is confined to the NB 6-2 (Weiss et al., 1998). In the head, at stage 9, *ind* is detected in an intermediate longitudinal ectodermal domain in the intercalary segment (*ind* is; Fig. 3C), and weakly in an intermediate ectodermal patch in the antennal segment (*ind* as; Fig. 3C) as well as in the deutocerebral NB Dd1 which develops from this patch (Fig. 3A). At the same stage, we observed a further signal in a dorsal ectodermal patch of the ocular region (*ind* oc, Fig. 3C). The ectodermal *ind* patches in the intercalary, antennal and ocular segments are both separate from each other and from the *ind* domain in the trunk (Fig. 3C,D,I,F). Interestingly, *ind* mRNA is significantly longer present in the ectoderm of the intercalary and mandibular segment, when compared with the antennal segment and the trunk ectoderm (data not shown). This presumably mirrors the delayed onset of neurogenesis in both segments (see also Urbach et al., 2003). Until stage 10, five NBs derive from the three *ind* patches: Td1, Td2, Td3, from the intercalary, Dd1 from the antennal and Ppd13 from the ocular *ind* patch (Fig. 3B,D). Subsequently, the ocular *ind* patch enlarges but never reaches the ocular *vnd* domain (Fig. 3F), and by stage 11 about four additional Ind expressing NBs (Pcd7, Pcd13, Ppd6, Ppd9) are identifiable (Fig. 3D,E).

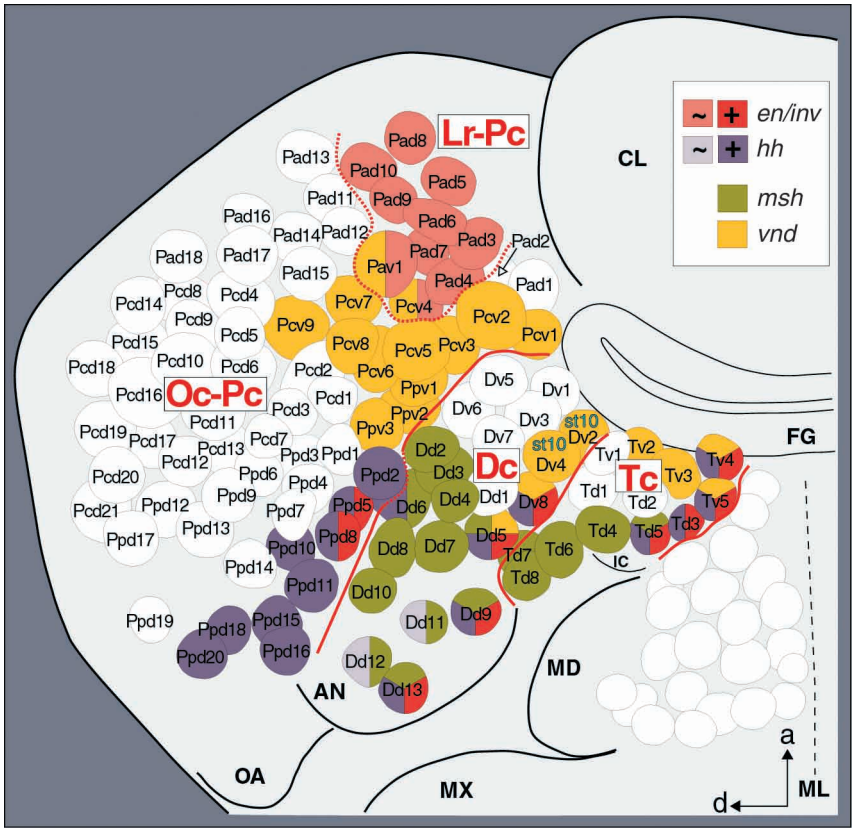


Fig. 4. Neuromeric model of the early embryonic brain. Based on the expression of the segment polarity genes *en/inv* and *hh* as well as the DV patterning genes *msh* and *vnd* we propose the pregnathal brain to consist of four neuromeres. Red lines indicate the borders between the tritocerebrum (Tc; comprising about 13 NBs), the deutocerebrum (Dc; comprising about 21 NBs), the ocular part of the protocerebrum (Oc-Pc; comprising about 60 NBs) and the labral part of the protocerebrum (Lr-Pc; comprising about 10 Inv-positive NBs). Note, the ventral part of the posterior border of the deutocerebrum is given by Dv2 and Dv4, which at stage 10 (st10) transiently express *vnd*. The neuromeric identity of Ppd2 is unclear; colour intensities indicate low (~) and high (+) expression levels of *en/inv* and *hh*. AN, IC, MD, MX, antennal, intercalary, mandibular and maxillary segment, respectively; CL, clypeolabrum; Dc, deutocerebrum; Lr-Pc, labral part of the protocerebrum; Oc-Pc, ocular part of the protocerebrum; Tc, tritocerebrum; FG, foregut; ML, ventral midline; OA, Bolwig organ/optic lobe anlagen.

muscle segment homeobox (*msh*) expression is first detected at the blastoderm stage in discontinuous patches in the dorsolateral part of the neuroectoderm, which later extend and form a bilateral longitudinal stripe (D'Alessio and Frasch, 1996); this domain gives rise to the lateral NBs of the ventral nerve cord (Isshiki et al., 1997). We detected at stage 7 *msh* expression anterior to the cephalic furrow (data not shown), which expands until stage 9 to cover, as a broad domain, the dorsal ectoderm of the intercalary and the antennal segment (Fig. 3C). As evidenced by Msh/Inv double labelling during stage 9 and stage 11, the anterior border of the *msh* domain coincides with the posterior border of the en hs (Fig. 3C,D,F,G). This suggests that *msh* expression in the pregnathal region is restricted to the intercalary and antennal segments, and matches the border between the antennal and ocular segment. This is further supported by Msh/*hh-lacZ* double labelling (data not shown) in stage 11 embryos, using *hh* as a marker for the posterior border of the ocular segment (for *hh* expression, see above and Figs 1, 2). All identified brain NBs delaminating from the dorsal intercalary and antennal neuroectoderm express *msh* (Fig. 3A,B,E,H). This suggests that during early neurogenesis, *msh* controls dorsal identities of the procephalic neuroectoderm and brain NBs, as was shown for the ventral nerve cord (Isshiki et al., 1997). In the ventral nerve cord, most glial precursor cells (glioblasts and neuroglioblasts) derive from the dorsal neuroectoderm (Schmidt et al., 1997), and express *msh* (Isshiki et al., 1997). In the intercalary segment of the early brain, we identified two glial precursors (Td4 and Td7) (see Urbach et al., 2003). Interestingly, both precursors are also located dorsally and express *msh*. At least until stage 11 we do not find *msh* expression in the preantennal segments.

Expression of DV patterning genes differs in the head and trunk neuroectoderm

Comparing the expression of DV patterning genes in the trunk and procephalic region we observed the following significant differences.

(1) Whereas *msh* is expressed in all segments of the trunk (Isshiki et al., 1997), it is not expressed in the preantennal head ectoderm (Fig. 3C-H).

(2) *ind* is expressed as a continuous stripe in the trunk, but forms three segmental patches in the procephalon. *ind* expression in the antennal segment appears to overlap with transient *vnd* expression [Fig. 3C; compare also with cell 'cluster 1' in McDonald et al. (McDonald et al., 1998)]. Yet, this ectodermal region gives rise to Dd1 which expresses *ind* but not *vnd* (Fig. 3A).

(3) The *msh* and *vnd* domains partially share a common border in the intercalary and antennal segment by stage 9 (Fig. 3C), and furthermore show a partial overlap in the antennal ectoderm by stage 10/11 (Fig. 3D,H). The En-positive Dd5 co-expresses *msh* and *vnd* (Fig. 3B,E,H,L), whereas co-expression of *msh* and *vnd* was not observed in NBs of the ventral nerve cord (McDonald et al., 1998).

(4) In the ocular segment the *ind* domain is separated from the *vnd* domain (Fig. 3C,D,F,I,), whereas in the trunk neuroectoderm these domains are adjacent to each other.

(5) *vnd* expression is dynamic and from stage 9 onwards is downregulated in parts of the antennal neuroectoderm and deutocerebral NBs (see *vnd* section).

(6) More than half of the total number of identified brain NBs do not express any of these DV patterning genes. Most of these NBs derive from the preantennal segments (Fig. 3A,B,E).

This implies that other, still unknown factors might be involved in the DV patterning of the anterior head neuroectoderm and protocerebrum.

Segmental boundaries in the early embryonic brain

With regard to the expression of the segment polarity genes *en*, *hh*, *wg* and *gsb-d*, as well as the DV patterning genes *msh* and *vnd*, we propose that the procephalic (pregnathal) neuroectoderm gives rise to four brain neuromeres: the tritocerebrum, the deutocerebrum, the ocular and the labral neuromere. These tightly fused neuromeres form a supra-oesophageal brain hemisphere on either side. The ocular and labral neuromeres represent the most prominent part of the brain which is traditionally referred to as the protocerebrum.

The detailed analysis of the dynamic expression of these genes in the procephalic neuroectoderm and in the identified brain NBs allows us to map the boundaries of the brain neuromeres (summarized in Fig. 4). The posterior border of the tritocerebrum is clearly represented by the *en*- and *hh* co-expressing NBs Tv4, Tv5, Td3, Td5. In the antennal and preantennal neuroectoderm the expression of *en*, *hh*, *wg* and *gsb-d* is largely restricted to intermediate and dorsal regions, and NBs deriving from there. Thus, regarding segment polarity genes, a clear demarcation of the antennal and preantennal neuromeres is only possible for the intermediate and dorsal, but not for the ventral domains. *vnd* is observed to be co-expressed with *en* in some tritocerebral (Tv5) and deutocerebral NBs (Dv8 and Dd5), located at intermediate DV positions. This is consistent with observations in the trunk, where *vnd* expression is dorsally expanded into each *en* domain in the neuroectoderm, as well as at the level of NBs (Chu et al., 1998). We therefore suggest that the (transiently) *vnd* expressing NBs Dv2 and Dv4, which follow Dd5 and Dv8 ventrally, demarcate the ventral part of the posterior border of the deutocerebrum. The intermediate part of this border is defined by the *en/hh/vnd*-co-expressing Dv8, Dd5, and the dorsal part by the *en*- and *hh*-co-expressing Dd9 and Dd13. For the posterior border of the ocular neuromere, we propose the following. Under the assumption that *vnd* expression also marks the posterior compartment in this neuromere, the *vnd* expressing NBs Pcv1, Pcv2, Pcv3, Ppv1, Ppv2 and Ppv3 would demarcate the ventral part of this border. The intermediate part is defined by the *en/hh*-co-expressing Ppd5 and Ppd8, and the dorsal part by the *Hh-lacZ*-positive NBs Ppd10, Ppd11, Ppd15 and Ppd16. Interestingly, the anterior border of the *msh* domain abutts exactly on the posterior ocular segmental border, indicating that *msh* expression is confined to the trito- and deutocerebrum. *inv* expression is observed in about 10 NBs deriving from the most anterior part of the protocerebral anlage, a region that corresponds to the En-positive 'dorsal hemispheres' (*en* dh) (Schmidt-Ott and Technau, 1992). We suggest that these NBs represent the neural correlate of the labral segment. The existence of a labral neuromere deriving from the *en* dh has already been discussed by Schmidt-Ott and Technau (Schmidt-Ott and Technau, 1992). This fourth brain neuromere seems to be of rudimentary character as it is confined to the posterior segmental compartment (considering that *en/inv* is normally expressed in the posterior compartment), and we did not find NBs anterior to *en* dh. Thus, the *wg* domain in the clypeolabral ectoderm, which is located immediately anterior to the *en* dh does not give rise to brain NBs (Fig. 2I,J). The existence of four brain neuromeres, in the spatial orientation shown, is furthermore substantiated by the segmental expression of other genes like *gsb-d* (Fig. 1D, Fig. 2J), *sloppy paired 1* and *ladybird* (see Urbach and Technau, 2003).

DISCUSSION

Reconstruction of neuromeric boundaries in the developing *Drosophila* brain

In previous papers, based on the expression of the segment polarity genes *en* and *wg*, and on the analysis of sensory structures in gap gene mutants, it was suggested that the *Drosophila* pregnathal head consisted of four segments, each contributing to the brain (Schmidt-Ott et al., 1994; Schmidt-Ott et al., 1995; Schmidt-Ott and Technau, 1992). However, a detailed description of related brain neuromeres was still lacking. In order to identify positional cues and segmental boundaries during early brain development, we analysed the expression of five different segment polarity genes (*en*, *wg*, *hh*, *mirr* and *gsb-d*) and three DV patterning genes (*msh*, *ind* and *vnd*) in the procephalic neurogenic region of the ectoderm, as well as in the entire population of brain NBs derived from this region. We focused our analysis on the developmental stages 9-11 for the following reasons: (1) the complex morphogenetic reorganization during the process of head involution has not yet taken place; (2) late stage 11 represents a phylotypic stage at which the head pattern is most clearly displayed (Jürgens and Hartenstein, 1993) and the full complement of brain NBs has formed (Urbach et al., 2003), except the primordia of the optic lobes (which develop after stage 11 and fuse secondarily with the brain) (Green et al., 1993); (3) it is possible to work on the level of identified brain NB; and (4) it is also possible to correlate gene expression in the outer ectoderm and in the evolving NBs.

Our data clearly support the view that the pregnathal head consists of four segments (antennal, intercalary, ocular and labral). Furthermore, we were able to attribute to each of the four pregnathal head segments a corresponding neuromere. All segment polarity genes are segmentally expressed in the pNR as well as in brain NBs, except *mirr*, the segmental expression of which is not overt. *wg* and *gsb-d* are partly overlapping, and are expressed anterior to the respective *en* domains, which are colocalized with *hh*. The expression of these genes is either mainly confined to intermediate and dorsal regions of the antennal and ocular segment (in case of *en*, *wg* and *gsb-d*) or is at least stronger (*hh*) in these parts of the pNR. Consequently, with regard to segment polarity genes there is a clear segmental demarcation, which is limited to intermediate and dorsal parts of the respective neuromeres, but it remains unclear in their ventral parts (except in the tritocerebrum). Surprisingly, we find that the DV patterning genes *vnd* and *msh* endorse a separation of brain neuromeres in AP axis. As outlined above, *vnd* expression demarcates the ventral part of the posterior border of the tritocerebrum, deutocerebrum and ocular neuromere, and *msh* the dorsal anterior border of the deutocerebrum. Thus, based on the expression of segment polarity genes (*en/inv*, *hh*) and DV patterning genes (*vnd*, *msh*) we provide for the first time a reconstruction of segmental boundaries in the developing brain on the level of identified cells (Fig. 4).

The protocerebrum is formed by the ocular segment and the posterior compartment of the labral segment

The segmental organization of the anterior head, in particular the origin of the labrum, the existence of a corresponding segment and its position at the anterior pole, are central issues of a long-lasting debate concerning head segmentation (e.g. Boyan et al., 2002; Haas et al., 2001; Jürgens and Hartenstein, 1993; Rogers and Kaufman, 1996; Schmidt-Ott et al., 1994; Scholz, 1998) (reviewed by Rempel, 1975). Consequently, the segmental origin of the protocerebrum, the largest and most anterior portion of the brain, has been a matter of debate and there is disagreement about whether it can be assigned to the labral and/or the ocular segment (equivalent to the acron).

en expression in the *en* dh has been attributed to the labral segment (Schmidt-Ott and Technau, 1992), the existence of which is further substantiated by PNS phenotypes in head gap mutants (Schmidt-Ott et al., 1994). We identify about 10 NBs that derive from this domain and weakly express *en*. Immediately anterior to the *en* dh, within the clypeolabral ectoderm, we find the genes *wg* (see also Schmidt-Ott and Technau, 1992), *gsb-d*, *lbe* and *slp1* (see Urbach and Technau, 2003) to be expressed, but we observed that these domains do not contribute to the brain. The spatial pattern of expression of these genes confirms the following: the anteroposterior orientation of a labral segment, as proposed by Schmidt-Ott and Technau (Schmidt-Ott and Technau, 1992); and a parasegmental character of the border between the *en* dh and the labral *wg* domain, supporting the view that the *en* dh is the *en*-expressing part of the labral segment. We therefore conclude that the protocerebrum consists of two neuromeres, a large ocular neuromere (comprising more than 60 NBs) and a smaller labral neuromere (comprising about 10 NBs). As *en* expression delimits the posterior compartment of each segment (Kornberg et al., 1985), the labral neuromere appears to be confined to the posterior compartment.

The protocerebrum develops prominent neuropile structures such as the central complex and the mushroom bodies (Hanesch et al., 1989; Strausfeld, 1976). On comparative morphological grounds, the protocerebrum in arthropods has been subdivided into the archicerebrum and prosocerebrum. Accordingly, the archicerebrum, which bears the optic lobes and mushroom bodies, belongs to the acron (or ocular segment) (Schmidt-Ott and Technau, 1992), and the prosocerebrum, which comprises the remainder of the protocerebrum (including the central complex and the neurosecretory cells of the pars intercerebralis) belongs to the labral segment (Larink, 1970; Malzacher, 1968; Scholl, 1969) (for a review, see Rempel, 1975). We identified the progenitor cells of the mushroom bodies to be part of the ocular neuromere (R.U. and G.M.T., unpublished), supporting the view that the mushroom bodies are indeed neuropil structures of the ocular segment or archicerebrum. Consequently, the identified labral NBs would be progenitors of neurones of the pars intercerebralis. This appears likely because the *en* dh during further embryogenesis becomes displaced in a brain region corresponding to the pars intercerebralis of postembryonic stages (Fig. 2M). In *Drosophila*, little is known about the embryonic origin of the central complex. In the grasshopper, it was recently documented that NBs in the pars intercerebralis contribute neurones to the central complex (Boyan and Williams, 1997). Taking into consideration that the identified labral NBs presumably represent the progenitors of cells of the pars intercerebralis and that the fundamental ‘bauplan’ of the brain is believed to be conserved among insects (Boyan et al., 1993; Nassif et al., 1998), we suggest that, in *Drosophila* progeny cells of labral NBs participate in the formation of the central complex.

The segmental character of the tritocerebrum and deutocerebrum is more conserved than that of the ocular and labral neuromere

In the trunk, the neuroectoderm and NB pattern of each hemisegment is subdivided by the activity of segment polarity genes into transverse rows and by the activity of DV patterning genes into longitudinal columns (for a review, see Skeath, 1999). We find that this orthogonal expression of segment polarity and DV patterning genes is principally conserved in the posterior part of the pregnathal head neuroectoderm and corresponding regions of the early brain, but becomes obscure towards anterior sites. The intercalary neuroectoderm and neuromere are subdivided by *en*, *hh*, *wg* and *gsb-d* expression into transverse-like rows and by *msh*, *ind* and *vnd* into longitudinal columns. Analysis of other genes that are segmentally expressed in the trunk CNS, e.g. *slp1* (Bhat et al., 2000; Cadigan et al., 1994a; Cadigan et al., 1994b), *ems* (Hartmann et al., 2000) and *lbe* (R.U. and G.M.T., unpublished), provides further support for the notion that the tritocerebrum behaves like a reduced trunk neuromere (see Urbach and Technau, 2003). Similarly, this orthogonal pattern of segment polarity and DV patterning gene expression appears to be essentially retained in the antennal neuroectoderm and deutocerebrum. However, it appears less conserved compared with the tritocerebrum because *en*, *wg* and *gsb-d* (and *slp1*) expression is confined to intermediate/dorsal sites, *ind* is restricted to one NB and *vnd* is only transiently expressed. The orthogonal expression pattern of both gene groups is to a minor extent, if at all, conserved in the posterior half of the ocular neuromere. Owing to the lack of *msh* expression, a dorsoventral polarity is less obvious and most ocular NBs do not express any DV patterning gene. Finally, conservation of this pattern is not evident in the labral segment. Although some segment polarity genes are expressed in the labral ectoderm, expression of DV patterning genes is missing (except for the two *vnd*-positive NBs, Pav1 and Pcv4, at the border to the ocular neuromere).

In this context, it is interesting to note that the head has been claimed to be composed of two distinct domains, an anterior terminal domain and a segmented region (Finkelstein and Perrimon, 1991). Both domains require high levels of Bicoid protein as an anterior determinant (Driever and Nüsslein-Volhard, 1988; Struhl et al., 1989), but the anterior terminal domain, which encompasses the labral segment and the acron (which is equivalent to the ocular segment) (Schmidt-Ott and Technau, 1992), is primarily specified by a signalling pathway mediated by the receptor tyrosine kinase TORSO (Klingler et al., 1988; Sprenger and Nüsslein-Volhard, 1992). Zygotic target genes which become activated by this signalling pathway (reviewed by Perrimon and Desplan, 1994) are the gap genes *hkb* and *tll* (Brönner et al., 1994; Pignoni et al., 1990). For *tll*, it has been shown that (part of) its anterior, blastodermal expression is necessary for the development of the protocerebrum, which is missing in *tll* mutants (Pignoni et al., 1990; Rudolph et al., 1997; Strecker et al., 1988). *tll* represses

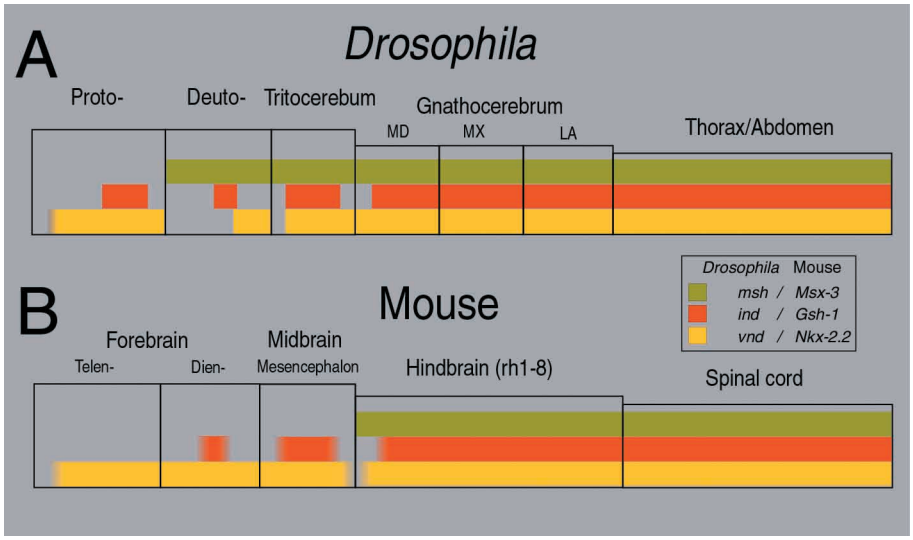


Fig. 5. Comparison of expression domains of DV patterning genes in the embryonic *Drosophila* and mouse CNS. (A,B) DV gene expression (as indicated by colour code) in the embryonic CNS of *Drosophila* at developmental stage 11 (A; compare Fig. 3) and mouse at ~10 days after gestation (B). Note, that anteriorly, the extent of expression is specific for each gene. Regional variabilities in the DV expansion of the respective expression domains is neglected. Mouse expression data are from Shimamura et al. (Shimamura et al., 1995) (*Nkx-2.2*), Valerius et al. (Valerius et al., 1995) (*Gsh-1*) and Shimeld et al. (Shimeld et al., 1996) (*Msx-3*). MD, MX, LA indicate mandibular, maxillary and labial neuromer; rh1-8, rhombomeres 1-8, respectively.

hb and *ftz* and may thus function in the head as an 'anti-segmentation' gene (Reinitz and Levine, 1990). We find that *tll* expression, which covers the ocular and labral neuroectoderm (the latter of which coincides with the region of the *en* dh) and emerging NBs (Urbach and Technau, 2003) (see also Rudolph et al., 1997), closely corresponds to that part of the early brain where segmental features are largely obscure. A coordinated, orthogonal expression of segment polarity and DV patterning genes within the ocular and labral neuroectoderm is not obvious, and the existence of putative serially homologous NBs in those regions of the brain is less evident (Urbach and Technau, 2003). This implies that *tll* might be a component crucial for the suppression of segmental characteristics in the ocular and labral neuromere. Furthermore, crossregulatory interactions among the segment polarity genes in the pregnathal head differ from those in the trunk and are unique for each pregnathal segment (Gallitano-Mendel and Finkelstein, 1997).

For a part of the segmented head (mandibular, intercalary and antennal) it was proposed that a combinatorial expression of the cephalic gap genes *otd*, *ems* and *buttonhead* (Finkelstein and Perrimon, 1990; Wimmer et al., 1993) mediates metamerization by acting directly on segment polarity genes, thereby omitting the intermediate function of pair rule genes (Cohen and Jürgens, 1990) (for a review, see Finkelstein and Perrimon, 1991). More recent data indicate that, in the segmental patterning of this head region, other (intermediate) regulators are involved. One of these is *collier*, which is already expressed in the blastoderm and is required for the formation of the intercalary segment. It is controlled by the combined activity of *ems* and *buttonhead*, and the pair rule gene *even skipped*, thus integrating inputs from both the head and trunk segmentation system (Crozatier et al., 1996; Crozatier et al., 1999). Such factors might help to explain that trunk specific segmental characteristics are more conserved in the intercalary and antennal neuroectoderm and NBs, when compared to the ocular and labral neuroectoderm and NBs.

Comparison of DV patterning gene expression in the *Drosophila* and vertebrate brain

In *Drosophila* the DV patterning genes subdivide the trunk neuroectoderm into longitudinal columns (for a review, see Cornell and Ohlen, 2000; Skeath, 1999); *vnd* is required for the specification of the ventral neuroectodermal column and NBs (Chu et al., 1998; Jimenez et al., 1995; McDonald et al., 1998; Mellerick and Nirenberg, 1995), *ind* and *msh* have analogous functions in the intermediate and dorsal neuroectodermal columns and NBs, respectively (D'Alessio and Frasch, 1996; Isshiki et al., 1997; Weiss et al., 1998). Remarkably, homologous genes are found to be expressed in the vertebrate neural plate and subsequently in the neural tube (Fig. 5). In the neural tube the order of expression along the DV axis is analogous to that of *Drosophila*: like *vnd*, the vertebrate homologs of the Nkx family are expressed in the ventral region; the *ind* homologs, *Gsh-1/2*, are expressed in the intermediate region; and the *msh* homologs, *Msx-1/2/3*, are expressed in the dorsal region of the neural tube (for a review, see Arendt and Nübler-Jung, 1999; Cornell and Ohlen, 2000).

As already discussed, we find these DV patterning genes to be expressed in the procephalic neuroectoderm and developing brain. Furthermore, we observe that, anteriorly, the extent of expression is specific for each gene: *msh* is confined to more posterior regions, and *vnd* expression extends into anterior regions of the brain. Moreover, the expression border of *msh* and *vnd* coincide with neuromeric borders. A comparison of the anteroposterior sequence of DV patterning gene expression in the early brain of *Drosophila*, with that published for the early mouse brain, reveals striking similarities (Fig. 5). *Msx3*, which presumably represents the ancestral *msh/Msx* gene, becomes restricted to the dorsal neural tube during later development (in contrast to *Msx1/2*) (Catron et al., 1996; Shimeld et al., 1996; Wang et al., 1996). The anterior border of the *Msx3* domain is positioned within the rostral region of the dorsal rhombencephalon (Wang et al., 1996), thus showing the shortest rostral extension of all vertebrate DV patterning genes. This displays analogy to *msh*, the expression domain of which coincides with the anterior border of the dorsal deutocerebrum, thus representing the shortest anterior extension of DV patterning genes in *Drosophila*. Mouse *Nkx2.2* extends ventrally into the most rostral areas of the forebrain (Price et al., 1992; Shimamura et al., 1995). *vnd* is

expressed ventrally in anterior parts of the ocular and labral protocerebrum. Thus, the expression of the respective homologs in both species displays the most anterior extension among DV patterning genes. Moreover, *Nkx2.2* expression in the mouse forebrain suggests that *Nkx2.2* may be involved in specifying diencephalic neuromeric boundaries (Price et al., 1992). Similarly, in *Drosophila*, dorsal expansions of the *vnd* domain appear to correspond to the tritocerebral and deutocerebral neuromeric boundaries.

Furthermore, *Drosophila ind* and its mouse homologue *Gsh1* show similarities in their expression in the early brain (Fig. 5). In the posterior parts of the *Drosophila* brain, *ind* is expressed in intermediate positions between *vnd* and *msh*. Likewise, in the posterior part of the mouse brain, *Gsh1* appears to be expressed in intermediate positions [see Fig. 4 by Valerius et al. (Valerius et al., 1995)], dorsally to *Nkx2.2* [for expression of *Nkx2.2*; see Fig. 3 by Shimamura et al. (Shimamura et al., 1995)], and in the hindbrain ventrally to *Msx3* [see Fig. 4 by Shimeld et al. (Shimeld et al., 1996)]. *Gsh1* has been shown to be expressed in discrete domains within the mouse hindbrain, midbrain (mesencephalon) and the most anterior domain in the posterior forebrain (diencephalon) (Valerius et al., 1995). Correspondingly, in *Drosophila* we find *ind* expression in restricted domains within the gnathocerebrum (R.U. and G.M.T., unpublished), the tritocerebrum, deutocerebrum and ocular part of the protocerebrum, demonstrating that the anteriormost extension of *ind* (and *Gsh1*) expression lies between that of *msh* and *vnd*.

Taken together, considering these similarities, we suggest that in the *Drosophila* and vertebrate early brain the expression of DV patterning genes is to some extent conserved, both along the DV axis (as suggested for the truncal parts of the *Drosophila* and mouse CNS) and along the AP axis. Furthermore, in *Drosophila* we observed that large parts of the anterodorsal procephalic neuroectoderm and NBs (more than 50% of all identified brain NBs) lack DV patterning gene expression. Likewise, in the vertebrate neural tube, gaps between the expression domains of DV patterning genes have been described, raising the possibility that other genes might fill in these gaps (Weiss et al., 1998). How DV fate is specified in the anterior and dorsal part of the *Drosophila* procephalic neuroectoderm, and if other genes are involved, remains to be clarified.

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REFERENCES

Arendt, D. and Nübler-Jung, K. (1999). Comparison of early nerve cord development in insects and vertebrates. *Development* **126**, 2309-2325.

Baker, N. E. (1988). Localization of transcripts from the *wingless* gene in whole *Drosophila* embryos. *Development* **103**, 289-298.

Baumgartner, S., Bopp, D., Burri, M. and Noll, M. (1987). Structure of two genes at the *gooseberry* locus related to the *paired* gene and their spatial expression during *Drosophila* embryogenesis. *Genes Dev.* **1**, 1247-1267.

Bhat, K. M. (1996). The *patched* signaling pathway mediates repression of *gooseberry* allowing neuroblast specification by *wingless* during *Drosophila* neurogenesis. *Development* **122**, 2921-2932.

Bhat, K. M. (1999). Segment polarity genes in neuroblast formation and identity specification during *Drosophila* neurogenesis. *BioEssays* **21**, 472-485.

Bhat, K. M., van Beers, E. H. and Bhat, P. (2000). *Sloppy paired* acts as the downstream target of *wingless* in the *Drosophila* CNS and interaction between *sloppy paired* and *gooseberry* inhibits *sloppy paired* during neurogenesis. *Development* **127**, 655-665.

Bier, E., Vaessin, H., Younger-Shepherd, S., Jan, L. Y. and Jan, Y. N. (1992). *deadpan*, an essential pan-neural gene in *Drosophila*, encodes a helix-loop-helix protein similar to the *hairy* gene product. *Genes Dev.* **6**, 2137-2151.

Bopp, D., Burri, M., Baumgartner, S., Frigerio, G. and Noll, M. (1986). Conservation of a large protein domain in the segmentation gene *paired* and in functionally related genes of *Drosophila*. *Cell* **47**, 1033-1040.

Boyan, G. S. and Williams, J. L. D. (1997). Embryonic development of the pars intercerebralis/central complex of the grasshopper. *Dev. Genes Evol.* **207**, 317-329.

Boyan, G. S. and Williams, J. L. D. (2000). Building the antennal lobe: *engrailed* expression reveals a contribution from protocerebral neuroblasts in the grasshopper *Schistocerca gregaria*. *Arthropod Struct. Dev.* **29**, 267-274.

Boyan, G. S., Williams, J. L. D. and Meier, T. (1993). Organization of the commissural fibers in the adult brain of the locust. *J. Comp. Neurol.* **332**, 358-377.

Boyan, G. S., Williams, J. L. D., Posser, S. and Bräunig, P. (2002). Morphological and molecular data argue for the labrum being non-apical, articulated, and the appendage of the intercalary segment in the locust. *Arthropod Struct. Dev.* **31**, 65-76.

Brand, M., Jarman, A. P., Jan, L. Y. and Jan, Y. N. (1993). *asense* is a *Drosophila* neural precursor gene and is capable of initiating sense organ formation. *Development* **119**, 1-17.

Broadus, J., Skeath, J. B., Spana, E. P., Bossing, T., Technau, G. M. and Doe, C. Q. (1995). New neuroblast markers and the origin of the aCC/pCC neurons in the *Drosophila* central nervous system. *Mech. Dev.* **53**, 393-402.

Brönner, G., Chu-LaGraff, Q., Doe, C. Q., Cohen, B., Weigel, D., Taubert, H. and Jäckle, H. (1994). *Sp1/egr*-like zinc-finger protein required for endoderm specification and germ-layer formation in *Drosophila*. *Nature* **369**, 664-668.

Bullock, T. H. and Horridge, G. A. (1965). *Structure and Function in the Nervous System of Invertebrates*. San Francisco, London: Freeman.

Cadigan, K. M., Grossniklaus, U. and Gehring, W. J. (1994a). Functional redundancy: the respective roles of the two *sloppy paired* genes in *Drosophila* segmentation. *Proc. Natl. Acad. Sci. USA* **91**, 6324-6328.

Cadigan, K. M., Grossniklaus, U. and Gehring, W. J. (1994b). Localized expression of *sloppy paired* protein maintains the polarity of *Drosophila* parasegments. *Genes Dev.* **8**, 899-913.

Campos-Ortega, J. A. (1993). Mechanisms of early neurogenesis in *Drosophila melanogaster*. *J. Neurobiol.* **24**, 1305-1327.

Campos-Ortega, J. A. (1998). The genetics of the *Drosophila achaete-scute* gene complex: a historical appraisal. *Int. J. Dev. Biol.* **42**, 291-297.

Campos-Ortega, J. A. and Hartenstein, V. (1997). *The Embryonic Development of* Drosophila melanogaster. Berlin, Heidelberg, New York: Springer Verlag.

Catron, K. M., Wang, H., Hu, G., Shen, M. M. and Abate-Shen, C. (1996). Comparison of *MSX-1* and *MSX-2* suggests a molecular basis for functional redundancy. *Mech. Dev.* **55**, 185-199.

Chu, H., Parras, C., White, K. and Jimenez, F. (1998). Formation and specification of ventral neuroblasts is controlled by *vnd* in *Drosophila* neurogenesis. *Genes. Dev.* **12**, 3613-3624.

Chu-LaGraff, Q. and Doe, C. Q. (1993). Neuroblast specification and formation regulated by *wingless* in the *Drosophila* CNS. *Science* **261**, 1594-1597.

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

Cornell, R. A. and Ohlen, T. V. (2000). *Vnd/nkx*, *ind/gsh*, and *msh/msx*: conserved regulators of dorsoventral neural patterning? *Curr. Opin. Neurobiol.* **10**, 63-71.

Crozatier, M., Valle, D., Dubois, L., Ibnsouda, S. and Vincent, A. (1996). *Collier*, a novel regulator of *Drosophila* head development, is expressed in a single mitotic domain. *Curr. Biol.* **6**, 707-718.

Crozatier, M., Valle, D., Dubois, L., Ibnsouda, S. and Vincent, A. (1999).

Head versus trunk patterning in the *Drosophila* embryo; *collier* requirement for formation of the intercalary segment. *Development* **126**, 4385-4394.

D'Alessio, M. and Frasch, M. (1996). *msh* may play a conserved role in dorsoventral patterning of the neuroectoderm and mesoderm. *Mech. Dev.* **58**, 217-231.

DiNardo, S., Kuner, J. M., Theis, J. and O'Farrell, P. H. (1985). Development of embryonic pattern in *Drosophila melanogaster* as revealed by accumulation of the nuclear *engrailed* protein. *Cell* **43**, 59-69.

Doe, C. Q. (1992). Molecular markers for identified neuroblasts and ganglion mother cells in the *Drosophila* central nervous system. *Development* **116**, 855-863.

Doe, C. Q. and Technau, G. M. (1993). Identification and cell lineage of individual neural precursors in the *Drosophila* CNS. *Trends Neurosci.* **16**, 510-514.

Driever, W. and Nüsslein-Volhard, C. (1988). The *bicoid* protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell* **54**, 95-104.

Duman-Scheel, M., Li, X., Orlov, I., Noll, M. and Patel, N. H. (1997). Genetic separation of the neural and cuticular patterning functions of *gooseberry*. *Development* **124**, 2855-2865.

Finkelstein, R. and Perrimon, N. (1990). The *orthodenticle* gene is regulated by *bicoid* and *torso* and specifies *Drosophila* head development. *Nature* **346**, 485-488.

Finkelstein, R. and Perrimon, N. (1991). The molecular genetics of head development in *Drosophila melanogaster*. *Development* **112**, 899-912.

Gallitano-Mendel, A. and Finkelstein, R. (1997). Novel segment polarity gene interactions during embryonic head development in *Drosophila*. *Dev. Biol.* **192**, 599-613.

Green, P., Hartenstein, A. Y. and Hartenstein, V. (1993). The embryonic development of the *Drosophila* visual system. *Cell Tissue Res.* **273**, 583-598.

Gutjahr, T., Patel, N. H., Li, X., Goodman, C. S. and Noll, M. (1993). Analysis of the *gooseberry* locus in *Drosophila* embryos: *gooseberry* determines the cuticular pattern and activates *gooseberry neuro*. *Development* **118**, 21-31.

Haas, M. S., Brown, S. J. and Beeman, R. W. (2001). Pondering the procephalon: the segmental origin of the labrum. *Dev. Genes Evol.* **211**, 89-95.

Hama, C., Ali, Z. and Kornberg, T. B. (1990). Region-specific recombination and expression are directed by portions of the *Drosophila engrailed* promoter. *Genes Dev.* **4**, 1079-1093.

Hanesch, U., Fischbach, K. F. and Heisenberg, M. (1989). Neuronal architecture of the central complex in *Drosophila melanogaster*. *Cell Tissue Res.* **257**, 343-366.

Hanström, B. (1928). *Vergleichende Anantomie des Nervensystems der wirbellosen Tiere*. Berlin: Springer.

Hartenstein, V. and Campos-Ortega, J. A. (1984). Early neurogenesis in wild-type *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **193**, 308-325.

Hartmann, B., Hirth, F., Walldorf, U. and Reichert, H. (2000). Expression, regulation and function of the homeobox gene *empty spiracles* in brain and ventral nerve cord development of *Drosophila*. *Mech. Dev.* **90**, 143-153.

Heisenberg, M. (1998). What do the mushroom bodies do for the insect brain? an introduction. *Learn. Mem.* **5**, 1-10.

Hirth, F., Therianos, S., Loop, T., Gehring, W. J., Reichert, H. and Furukubo-Tokunaga, K. (1995). Developmental defects in brain segmentation caused by mutations of the homeobox genes *orthodenticle* and *empty spiracles* in *Drosophila*. *Neuron* **15**, 769-778.

Isshiki, T., Takeichi, M. and Nose, A. (1997). The role of the *msh* homeobox gene during *Drosophila* neurogenesis: implication for the dorsoventral specification of the neuroectoderm. *Development* **124**, 3099-3109.

Jagla, K., Jagla, T., Heitzler, P., Dretzen, G., Bellard, F. and Bellard, M. (1997). *ladybird*, a tandem of homeobox genes that maintain late *wingless* expression in terminal and dorsal epidermis of the *Drosophila* embryo. *Development* **124**, 91-100.

Jimenez, F., Martin-Morris, L. E., Velasco, L., Chu, H., Sierra, J., Rosen, D. R. and White, K. (1995). *vnd*, a gene required for early neurogenesis of *Drosophila*, encodes a homeodomain protein. *EMBO J.* **14**, 3487-3495.

Jürgens, G. and Hartenstein, V. (1993). The terminal regions of the body pattern. In *The Development of* Drosophila melanogaster (ed. C. M. Bate and A. Martinez-Arias), pp. 687-746. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Jürgens, G., Lehmann, R., Schradin, M. and Nüsslein-Volhard, C. (1986). Segmental organisation of the head in the embryo of *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **193**, 283-295.

Klingler, M., Erdelyi, M., Szabad, J. and Nüsslein-Volhard, C. (1988). Function of *torso* in determining the terminal anlagen of the *Drosophila* embryo. *Nature* **335**, 275-277.

Kornberg, T., Siden, I., O'Farrell, P. and Simon, M. (1985). The *engrailed* locus of *Drosophila*: in situ localization of transcripts reveals compartment-specific expression. *Cell* **40**, 45-53.

Larink, O. (1970). Die Kopfentwicklung von *Lepisma saccharina* L. (Insecta, Thysanura). *Z. Morphol. Tiere* **67**, 1-15.

Malzacher, P. (1968). Die Embryogenese des Gehirns paurometaboler Insekten. Untersuchungen an *Carausius morosus* und *Periplaneta americana*. *Z. Morphol. Tiere* **62**, 103-161.

McDonald, J. A., Holbrook, S., Isshiki, T., Weiss, J., Doe, C. Q. and Mellerick, D. M. (1998). Dorsoventral patterning in the *Drosophila* central nervous system: the *vnd* homeobox gene specifies ventral column identity. *Genes Dev.* **12**, 3603-3612.

McNeill, H., Yang, C. H., Brodsky, M., Ungos, J. and Simon, M. A. (1997). *mirror* encodes a novel PBX-class homeoprotein that functions in the definition of the dorsal-ventral border in the *Drosophila* eye. *Genes Dev.* **11**, 1073-1082.

Mellerick, D. M. and Nirenberg, M. (1995). Dorsal-ventral patterning genes restrict *NK-2* homeobox gene expression to the ventral half of the central nervous system of *Drosophila* embryos. *Dev. Biol.* **171**, 306-316.

Mohler, J., Mahaffey, J. W., Deutsch, E. and Vani, K. (1995). Control of *Drosophila* head segment identity by the *bZIP* homeotic gene *cnc*. *Development* **121**, 237-247.

Mohler, J. and Vani, K. (1992). Molecular organization and embryonic expression of the *hedgehog* gene involved in cell-cell communication in segmental patterning of *Drosophila*. *Development* **115**, 957-971.

Nassif, C., Noveen, A. and Hartenstein, V. (1998). Embryonic development of the *Drosophila* brain. I. Pattern of pioneer tracts. *J. Comp. Neurol.* **402**, 10-31.

Ouellette, R. J., Valet, J. P. and Cote, S. (1992). Expression of *gooseberry-proximal* in the developing nervous system responds to cues provided by segment polarity genes. *Roux's Arch. Dev. Biol.* **201**, 157-168.

Patel, N. H. (1994). Imaging neuronal subsets and other cell types in whole mount *Drosophila* embryos and larvae using antibody probes. In *Methods in Cell bBology.* Drosophila melanogaster*: Practical Uses in Cell Biology.* Vol. 44 (ed. L. S. B. Goldstein and E. Fyrberg). New York: Academic Press.

Patel, N. H., Martin-Blanco, E., Coleman, K. G., Poole, S. J., Ellis, M. C., Kornberg, T. B. and Goodman, C. S. (1989). Expression of *engrailed* proteins in arthropods, annelids, and chordates. *Cell* **58**, 955-968.

Perrimon, N. and Desplan, C. (1994). Signal transduction in the early *Drosophila* embryo: when genetics meets biochemistry. *Trends Biochem. Sci.* **19**, 509-513.

Pignoni, F., Baldarelli, R. M., Steingrimsson, E., Diaz, R. J., Patapoutian, A., Merriam, J. R. and Lengyel, J. A. (1990). The *Drosophila* gene *tailless* is expressed at the embryonic termini and is a member of the steroid receptor superfamily. *Cell* **62**, 151-163.

Plickert, G., Gajewski, M., Gehrke, G., Gausepohl, H., Schlossherr, J. and Ibrahim, H. (1997). Automated in situ detection (AISD) of biomolecules. *Dev. Genes Evol.* **207**, 362-367.

Poole, S. J., Kauvar, L. M., Drees, B. and Kornberg, T. (1985). The *engrailed* locus of *Drosophila*: structural analysis of an embryonic transcript. *Cell* **40**, 37-43.

Price, M., Lazzaro, D., Pohl, T., Mattei, M. G., Ruther, U., Olivo, J. C., Duboule, D. and di Lauro, R. (1992). Regional expression of the homeobox gene *Nkx-2.2* in the developing mammalian forebrain. *Neuron* **8**, 241-255.

Reinitz, J. and Levine, M. (1990). Control of the initiation of homeotic gene expression by the gap genes *giant* and *tailless* in *Drosophila*. *Dev. Biol.* **140**, 57-72.

Rempel, J. G. (1975). The evolution of the insect head: an endless dispute. *Quaestiones Entomologicae* **11**, 7-25.

Richter, S., Hartmann, B. and Reichert, H. (1998). The *wingless* gene is required for embryonic brain development in *Drosophila*. *Dev. Genes Evol.* **208**, 37-45.

Rogers, B. T. and Kaufman, T. C. (1996). Structure of the insect head as revealed by the EN protein pattern in developing embryos. *Development* **122**, 3419-3432.

Rudolph, K. M., Liaw, G. J., Daniel, A., Green, P., Courey, A. J., Hartenstein, V. and Lengyel, J. A. (1997). Complex regulatory region

mediating *tailless* expression in early embryonic patterning and brain development. *Development* **124**, 4297-4308.

Schmidt, H., Rickert, C., Bossing, T., Vef, O., Urban, J. and Technau, G. M. (1997). The embryonic central nervous system lineages of *Drosophila melanogaster*. II. Neuroblast lineages derived from the dorsal part of the neuroectoderm. *Dev. Biol.* **189**, 186-204.

Schmidt-Ott, U. and Technau, G. M. (1992). Expression of *en* and *wg* in the embryonic head and brain of *Drosophila* indicates a refolded band of seven segment remnants. *Development* **116**, 111-125.

Schmidt-Ott, U. and Technau, G. M. (1994). Fate-mapping in the procephalic region of the embryonic *Drosophila* head. *Roux's Arch. Dev. Biol.* **203**, 367-373.

Schmidt-Ott, U., Gonzalez-Gaitan, M., Jäckle, H. and Technau, G. M. (1994). Number, identity, and sequence of the *Drosophila* head segments as revealed by neural elements and their deletion patterns in mutants. *Proc. Natl. Acad. Sci. USA* **91**, 8363-8367.

Schmidt-Ott, U., Gonzalez-Gaitan, M. and Technau, G. M. (1995). Analysis of neural elements in the head-mutant *Drosophila* embryos suggests a segmental origin of the optic lobes. *Roux's Arch. Dev. Biol.* **205**, 31-44.

Scholl, G. (1969). Die Embryonalentwicklung des Kopfes und Prothorax von *Carausius morosus* Br. (Insecta, Phasmida). *Z. Morphol. Tiere* **65**, 1-142.

Scholz, G. (1998). Cleavage, germ band formation and head segmentation: the ground pattern of the Euarthropoda. In *Arthropod relationships* (ed. R. A. Fortey and R. H. Thomas), pp. 317-332, London: Chapman & Hall.

Shao, X., Koizumi, K., Nosworthy, N., Tan, D. P., Odenwald, W. and Nirenberg, M. (2002). Regulatory DNA required for *vnd/NK-2* homeobox gene expression pattern in neuroblasts. *Proc. Natl. Acad. Sci. USA* **99**, 113-117.

Shimamura, K., Hartigan, D. J., Martinez, S., Puelles, L. and Rubenstein, J. L. (1995). Longitudinal organization of the anterior neural plate and neural tube. *Development* **121**, 3923-3933.

Shimeld, S. M., McKay, I. J. and Sharpe, P. T. (1996). The murine homeobox gene *Msx-3* shows highly restricted expression in the developing neural tube. *Mech. Dev.* **55**, 201-210.

Skeath, J. B. (1999). At the nexus between pattern formation and cell-type specification: the generation of individual neuroblast fates in the *Drosophila* embryonic central nervous system. *BioEssays* **21**, 922-931.

Skeath, J. B., Zhang, Y., Holmgren, R., Carroll, S. B. and Doe, C. Q. (1995). Specification of neuroblast identity in the *Drosophila* embryonic central nervous system by *gooseberry-distal*. *Nature* **376**, 427-430.

Sprenger, F. and Nüsslein-Volhard, C. (1992). *Torso* receptor activity is regulated by a diffusible ligand produced at the extracellular terminal regions of the *Drosophila* egg. *Cell* **71**, 987-1001.

Strausfeld, N. J. (1976). *Atlas of an Insect Brain*. Heidelberg, Germany: Springer.

Strauss, R. and Heisenberg, M. (1993). A higher control center of locomotor behavior in the *Drosophila* brain. *J. Neurosci.* **13**, 1852-1861.

Strecker, T. R., Merriam, J. R. and Lengyel, J. A. (1988). Graded requirement for the zygotic terminal gene, *tailless*, in the brain and tail region of the *Drosophila* embryo. *Development* **102**, 721-734.

Struhl, G., Struhl, K. and Macdonald, P. M. (1989). The gradient morphogen *bicoid* is a concentration-dependent transcriptional activator. *Cell* **57**, 1259-1273.

Tabata, T., Eaton, S. and Kornberg, T. B. (1992). The *Drosophila hedgehog* gene is expressed specifically in posterior compartment cells and is a target of *engrailed* regulation. *Genes Dev.* **6**, 2635-2645.

Tautz, D. and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. *Chromosoma* **98**, 81-85.

Uhler, J., Garbern, J., Yang, L., Kamholz, J. and Mellerick, D. M. (2002). *Nk6*, a novel *Drosophila* homeobox gene regulated by *vnd*. *Mech. Dev.* **116**, 105-116.

Urbach, R. and Technau, G. M. (2003). Molecular markers for identified neuroblasts in the developing brain of *Drosophila*. *Development* **130**, 3621-3637.

Urbach, R., Schnabel, R. and Technau, G. M. (2003). The pattern of neuroblast formation, mitotic domains, and proneural gene expression during early brain development in *Drosophila*. *Development* **130**, 3589-3606.

Valerius, M. T., Li, H., Stock, J. L., Weinstein, M., Kaur, S., Singh, G. and Potter, S. S. (1995). *Gsh-1*: a novel murine homeobox gene expressed in the central nervous system. *Dev. Dyn.* **203**, 337-351.

van den Heuvel, M., Nusse, R., Johnston, P. and Lawrence, P. A. (1989). Distribution of the *wingless* gene product in *Drosophila* embryos: a protein involved in cell-cell communication. *Cell* **59**, 739-749.

Wang, W., Chen, X., Xu, H. and Lufkin, T. (1996). *Msx3*: a novel murine homologue of the *Drosophila msh* homeobox gene restricted to the dorsal embryonic central nervous system. *Mech. Dev.* **58**, 203-215.

Weiss, J. B., von Ohlen, T., Mellerick, D. M., Dressler, G., Doe, C. Q. and Scott, M. P. (1998). Dorsoventral patterning in the *Drosophila* central nervous system: the *intermediate neuroblasts defective* homeobox gene specifies intermediate column identity. *Genes Dev.* **12**, 3591-3602.

Wimmer, E. A., Jäckle, H., Pfeifle, C. and Cohen, S. M. (1993). A *Drosophila* homologue of human *Sp1* is a head-specific segmentation gene. *Nature* **366**, 690-694.

Younossi-Hartenstein, A., Nassif, C., Green, P. and Hartenstein, V. (1996). Early neurogenesis of the *Drosophila* brain. *J. Comp. Neurol.* **370**, 313-329.

Zhang, Y., Ungar, A., Fresquez, C. and Holmgren, R. (1994). Ectopic expression of either the *Drosophila gooseberry-distal* or *proximal* gene causes alterations of cell fate in the epidermis and central nervous system. *Development* **120**, 1151-1161.