COMPARTMENTS AND THEIR BOUNDARIES IN VERTEBRATE BRAIN DEVELOPMENT

Clemens Kiecker and Andrew Lumsden

Abstract | Fifteen years ago, cell lineage restriction boundaries were discovered in the embryonic vertebrate hindbrain, subdividing it into a series of cell-tight compartments (known as rhombomeres). Compartition, together with segmentally reiterative neuronal architecture and the nested expression of *Hox* genes, indicates that the hindbrain has a truly metameric organization. This finding initiated a search for compartments in other regions of the developing brain. The results of recent studies have clarified where compartment boundaries exist, have shed light on molecular mechanisms that underlie their formation and have revealed an important function of these boundaries: the positioning and stabilization of local signalling centres.

COMPARTMENT
A module of the embryo that consists of polyclonally-related cells that do not mix with cells from neighbouring compartments.

PATTERNING
A developmental process during which cells that are initially equal acquire different identities.

BAUPLAN
German for 'construction plan'.

Medical Research Council Centre for Developmental Neurobiology, King's College London, Guy's Hospital Campus, London SE1 1UL, UK. Correspondence to A.L. e-mail: andrew.lumsden@kcl.ac.uk
doi:10.1038/nrn1702

The importance of cell lineage restriction boundaries was recognized in studies on invertebrate development well before the era of molecular biology. In 1973, both the abdomen and the wing anlage of insect embryos were found to be segregated into cellular COMPARTMENTS by boundaries that cells do not cross, thereby imposing lineage restriction on groups of cells[1–3]. It was postulated that compartment boundaries serve a dual function during development — first, by preventing the intermingling of cells that are fated to contribute to different parts of the embryo and, second, by providing positional information to flanking cell populations. Therefore, boundaries are essential to coordinate growth and PATTERNING in an embryo that rapidly increases in size and complexity. This concept has since found support through studies of various mutant strains of the fruitfly *Drosophila melanogaster*, in which specific growth and/or patterning defects can be traced back to impaired boundary formation[4–8].

At first sight, the vertebrate nervous system does not seem to have much in common with the BAUPLAN of an insect embryo. However, in 1990 the segment-like rhombomeres of the embryonic hindbrain, which had long been known as morphological entities[9], were found to be lineage-restricted compartments[10]. *Hox* GENES are expressed in the hindbrain in a nested fashion and their borders of expression coincide with rhombomere boundaries[11], much like their nested expression in the *D. melanogaster* embryo during the regulation of segmental identity. These observations indicated that the hindbrain is a truly segmented region, and this triggered a search for underlying SEGMENTATION in other parts of the developing brain.

Here, we briefly review the evidence for a segmental organization of the hindbrain and discuss a prevailing model for forebrain segmentation. Many recent studies have used combinations of classic and novel techniques in various vertebrate model systems in attempts to identify and characterize neural lineage restriction boundaries, and we critically compare their different approaches. One of the principal conclusions of these studies is that many previously assumed intersegmental boundaries in the forebrain are not lineage restriction boundaries, which indicates that large parts of the forebrain develop in an unsegmented fashion. We further show — by analogy to *D. melanogaster* — that one of the main functions of boundaries during brain development is to position and stabilize local signalling centres that function by informing cells in adjacent territories of their position and fate. Finally, we summarize recent work that has identified some of the molecular players that are involved in the

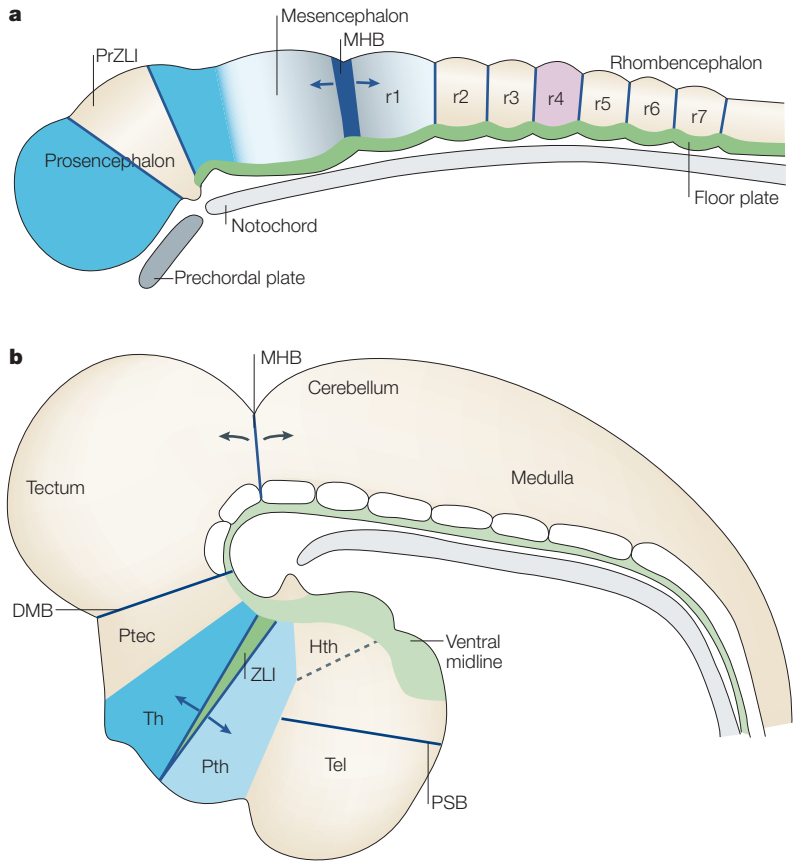

Figure 1 | **Boundaries and local signalling centres in the developing vertebrate neural tube.** Lateral view of embryonic avian brain. **a** | Hamburger–Hamilton stage 13 (HH 13), anterior to the left, dorsal to the top. At this stage, cell lineage restriction (dark blue) is found along the anterior and posterior borders of the presumptive zona limitans intrathalamica (PrZLI), at the midbrain–hindbrain boundary (MHB) and between rhombomeres (r1–7). Major signalling centres are the MHB, rhombomere boundaries, rhombomere 4 (r4) and the floor plate. Arrows represent bidirectional signalling from the MHB. Also shown are the prechordal plate and the notochord, which are two non-neural signalling centres that influence dorsoventral patterning of the neural tube. **b** | Stage HH 24. Cell lineage restriction is in force at the pallial–subpallial boundary (PSB), anterior and posterior to the zona limitans intrathalamica (ZLI), at the diencephalon–midbrain boundary (DMB) and between former hindbrain rhombomeres in the proliferating zone (not shown). Major local signalling centres at this stage are the ZLI, the MHB and the ventral midline. Arrows represent bidirectional signalling from the MHB and the ZLI. Hth, hypothalamus; Ptec, pretectum; Pth, prethalamus; Tel, telencephalon; Th, thalamus.

establishment of boundaries, and relate these results to classic models for tissue separation.

Hindbrain segmentation

The embryonic neuroepithelium is characterized morphologically by a series of constrictions and bulges, many of which appear only transiently during development (FIG. 1). For a long time the significance of these so-called neuromeres remained enigmatic, although the possibility was sporadically raised that they reflect some rudimentary form of segmentation[9]. The developing hindbrain forms a particularly distinctive series of seven or eight neuromeres (FIG. 1a), and several findings support the idea that these rhombomeres (r) are true segments. First, proliferation, neurogenesis and axonal projections are arranged in a reiterative fashion in successive rhombomeres[12–16]; second, rhombomeres are compartments that are separated by cell lineage restriction boundaries[10]; third, rhombomere boundaries show reduced proliferation and express specific molecular markers[13,17–21]; and fourth, orthologues of *D. melanogaster Hox* genes are expressed in an ordered and nested manner in the hindbrain, and their borders of expression coincide with rhombomere boundaries[11] (FIG. 2). Therefore, hindbrain architecture bears a striking resemblance to the *D. melanogaster* embryo body plan, which is established through a cascade of genes that drive progressive anteroposterior subregionalization of the embryo, resulting in a segmented larva in which every segment will give rise to a specific part of the adult fly. The positional identity of each segment is defined by the combinatorial expression of HOMEOTIC SELECTOR GENES such as the *Hox* genes.

The importance of *Hox* genes in regulating rhombomere identity has been highlighted by gain- and loss-of-function studies of several *Hox* genes, and most particularly of *Hoxb1*, a gene that is uniquely expressed in a single rhombomere, r4 (FIG. 2). This rhombomere shows characteristics of r2 in *Hoxb1*-deficient mice; specifically, facial motor neurons born in r4 fail to migrate caudally into r6 and vestibuloacoustic neurons fail to migrate to the contralateral side of r4. Instead, both types of neuron migrate dorsolaterally like the r2-specific trigeminal motor neurons[22]. This phenotype is also seen in zebrafish embryos that lack *hoxb1a* function[23]. Conversely, ectopic expression of *HOXB1* in the r2 of chick embryos leads trigeminal motor neurons to adopt r4-like characteristics and project into the second BRANCHIAL ARCH, like the normal facial motor neurons of r4 (REF. 24). These studies indicate that *Hoxb1* specifies aspects of r4 identity in a selector gene-like fashion: both removal of the selector from its segment and ectopic expression in another segment result in homeotic transformations in which one segment adopts the phenotype of the other. Similarly, facial and trigeminal-like neurons can be induced in r1, which is normally devoid of motor neurons, following ectopic expression of *HOXB1* and *HOXA2*, respectively[25].

Together, these findings indicate that the embryonic hindbrain satisfies the criteria for a segmented structure: the continuous neuroepithelium is subdivided into transverse cell lineage-restricted compartments that are serially arrayed along its anteroposterior axis and the positional identity of which is regulated, at least in part, by the differential expression of selector (*Hox*) genes. The repetitiveness of neuronal architecture in successive rhombomeres indicates that the hindbrain has a truly METAMERIC organization. However, it should be noted that this segmentation is incomplete, as no lineage restriction has been detected along the FLOOR PLATE (which is reflected by a lack of morphological segmentation in this region[10]). By contrast, segmental organization might be present in its dorsal counterpart, the roof plate, as revealed by a genetic labelling strategy in mice[26]. Furthermore, a small percentage of cells are able to cross rhombomeric lineage restrictions[27]. The biological significance of this apparent 'leakiness'

Hox GENES
A family of developmental regulator genes present in all animal phyla that are arranged in clusters in the genome and encode transcription factors with a DNA-binding homeobox.

SEGMENTATION
The process of dividing an embryonic region into semi-independent, cell lineage-restricted compartments — a way of organizing embryogenesis of a large region by subdividing it into a repetitive series of small fields.

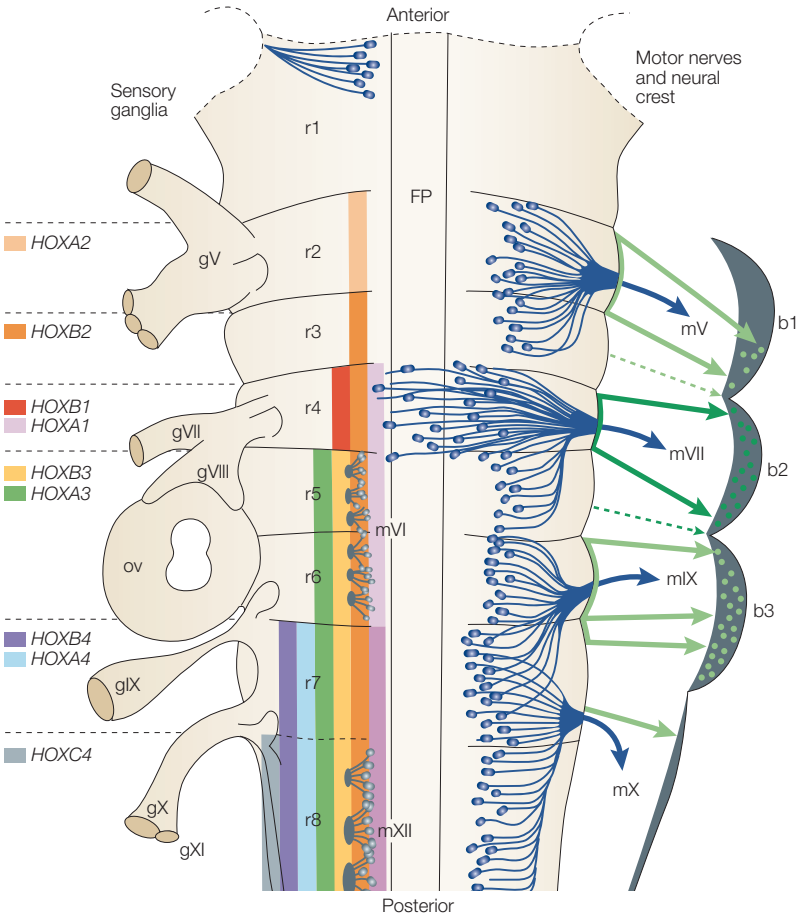

Figure 2 | **Hindbrain segmentation.** Schematic representation of a vertebrate (chick) hindbrain in dorsal view without the roof plate. The reiterative formation of motor nuclei and the exit points of their efferent nerves from rhombomeres 2, 4, 6 and 7 (r2, r4, r6 and r7) are indicated on the right side. The trigeminal (mV), facial (mVII) and glossopharyngeal cranial (mIX) nerves project into the first (b1), second (b2) and third (b3) branchial arches, respectively, and the vagus nerve (mX) innervates a large part of the body. Neural crest cells from the corresponding rhombomeres also populate the periphery in a segmental fashion (green arrows). The positions of the cranial sensory ganglia (gV and gVII–gXI) and the otic vesicles (ov) are indicated on the left side. The segmental nested expression of *HOX* genes is colour-coded. FP, floor plate; mVI, mXII, somatic motor neurons.

might be that although lineage restriction remains in effect up to late stages of neurogenesis in the proliferating VENTRICULAR ZONE, postmitotic neurons of the MANTLE ZONE are able to cross rhombomere boundaries during programmed neuronal migration[28]. This indicates that cell-tight boundaries might only be required in proliferating cell populations with labile cell fates that are still subject to specification; positional restriction is likely to become dispensable for postmitotic cells, as their fates are specified.

HOMEOTIC SELECTOR GENES Genes, such as those of the *Hox* family, that determine the positional identity of the embryonic region in which they are expressed. Absence or ectopic misexpression of such genes results in the lack or duplication of this region (homeotic transformation).

Forebrain boundaries and the prosomeric model

The forebrain is structurally much more complicated than the hindbrain, but it is also characterized by the appearance of transient bulges and constrictions of the neuroepithelium (FIG. 1). The more detailed understanding of hindbrain segmentation revived older efforts to describe the forebrain in the context of neuromery[9], and various models for forebrain segmentation were developed during the 1990s. In 1993, Figdor and Stern proposed a subdivision of the posterior part of the forebrain, the diencephalon, into four neuromeres, D1–D4, on the basis of an analysis of morphology, differential distribution of neuronal antigens, axonal architecture, marker gene expression and lineage-labelling experiments in chick embryos[29]. In the same year, cell-labelling experiments in cultured mouse embryos revealed a dorsoventral lineage restriction boundary between the cortex and the lateral ganglionic eminence (pallial–subpallial boundary, PSB) within the telencephalon, the anterior part of the forebrain (FIG. 1b). Similar to rhombomere boundaries, lineage restriction is only effective in the ventricular zone at the PSB, whereas postmitotic neurons are able to freely cross this boundary in the mantle zone[30].

The nested expression of *Hox* genes has been one of the principal arguments for a segmented organization of the vertebrate hindbrain (FIG. 2). *Hox* genes are not expressed anterior to r2, but other transcription factor-encoding genes, many of which are orthologues of genes that regulate anterior development in *D. melanogaster*, show highly localized expression patterns in the forebrain–midbrain area (most notably, members of the distalless (*Dlx*), empty spiracles (*Emx*), forkhead (*Fox*), orthodenticle (*Otx*), paired (*Pax*) and sine oculis (*Six*) families)[31,32]. In the early 1990s, Puelles, Rubenstein and co-workers proposed a NEUROMERIC ORGANIZATION of the entire forebrain on the basis of the differential expression of these neural marker genes combined with morphological considerations. According to their 'prosomeric model', the forebrain consists of six transverse subdivisions, known as prosomeres, the posterior three of which (p1–p3) represent subdivisions of the diencephalon, whereas the anterior three (p4–p6) subdivide the secondary prosencephalon (hypothalamus and telencephalon)[33]. This model has proved useful as it provides a topographical framework for studies on forebrain development.

However, the expression domains of various forebrain markers were found to be highly dynamic with respect to morphological forebrain subdivisions[34]. A recent fate-mapping study performed in our laboratory revealed that cells are able to cross the proposed boundary between the synencephalon (prospective pretectum, p1) and the parencephalon (prospective thalamus and prethalamus, p2 and p3) as well as the boundary between the prethalamus (p3) and the secondary prosencephalon[35]. Furthermore, no evidence for anteroposterior lineage restriction has been found anterior to the p2/p3 boundary[36–38]. Finally, no uniform set of boundary markers is expressed at all of the proposed interprosomeric boundaries[35]. Therefore, the only true cell lineage restriction boundaries in the forebrain are the PSB, the diencephalon–midbrain boundary (DMB)[39], and the interface between the thalamic and the prethalamic primordia, the zona limitans intrathalamica (ZLI; FIG. 1b). A revised prosomeric model that takes these findings into account has since been published[40].

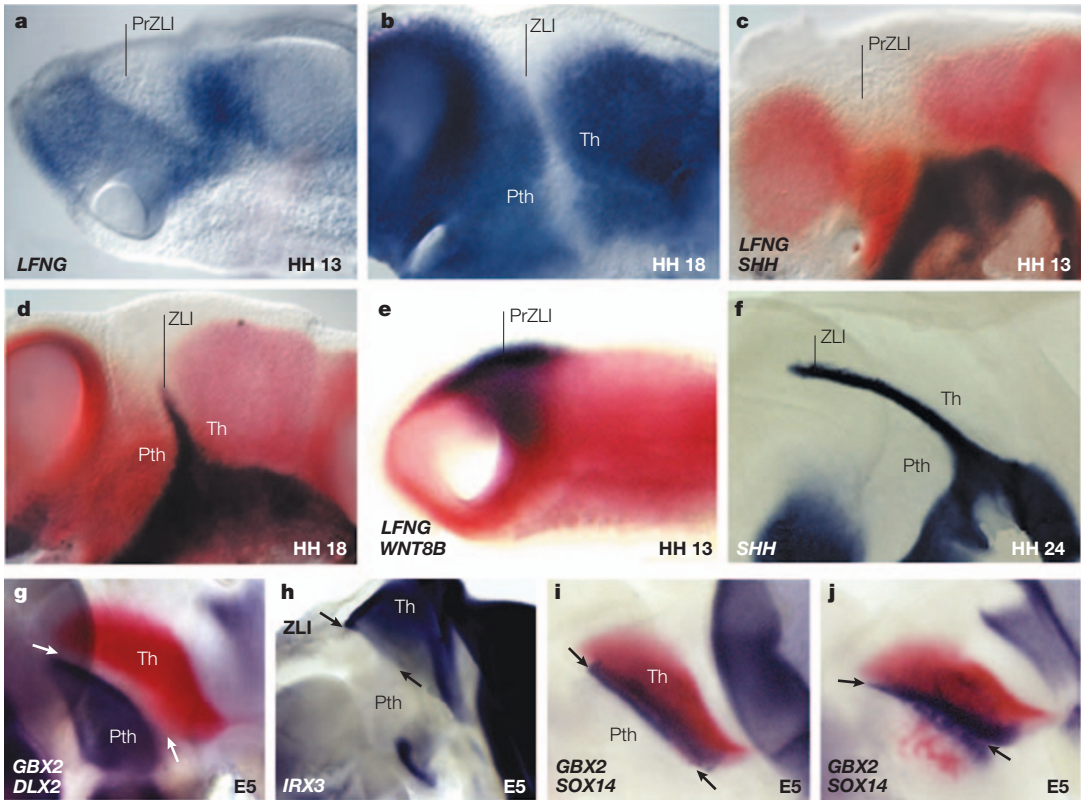

Figure 3 | **Formation of and signalling from the zona limitans intrathalamica.** Lateral views of embryonic chick brains (anterior to the left, dorsal to the top); gene expression has been revealed by *in situ* hybridization. **a** | At Hamburger–Hamilton stage 13 (HH 13), lunatic fringe (*LFNG*) is expressed throughout the prosencephalon except in a wedge-shaped area (presumptive zona limitans intrathalamica; PrZLI) within the presumptive diencephalon. **b** | At later stages, the *LFNG*-negative area has narrowed relative to the other parts of the forebrain, and forms a narrow transverse band of cells. Pth, prethalamus; Th, thalamus; ZLI, zona limitans intrathalamica. **c** | Sonic hedgehog (*SHH*) is expressed exclusively along the ventral midline of the neural tube at HH 13, but once the wedge has narrowed (HH 18; **d**) a peak of *SHH* expression extends dorsally into the *LFNG*-negative area. **e** | *WNT8B* is expressed in the *LFNG*-negative wedge. **f** | *SHH* marks the ZLI at later stages of neural development. **g** | *DLX2* (distalless homeobox 2) expression (purple) marks the prethalamus and *GBX2* (gastrulation brain homeobox 2) expression (red) marks the thalamus. **h** | *IRX3* (iroquois homeobox 3) is expressed posteriorly to the ZLI. **i** | *SOX14* (high mobility group (HMG) box transcription factor 14) expression (purple) flanks the ZLI posteriorly in a narrower domain than *GBX2* expression (red). **j** | Ectopic expression of *IRX3* in the prethalamus results in mirrored expression of the thalamus markers *GBX2* and *SOX14* in the prethalamic area and the downregulation of the prethalamic marker *DLX2* (not shown). E5, embryonic day 5. Panels **a**–**d** reproduced, with permission, from REF. 41 © (2001) Macmillan Magazines Ltd. Panels **f**–**j** reproduced, with permission, from REF. 67 © (2004) Macmillan Magazines Ltd.

BRANCHIAL ARCHES
(Also called pharyngeal arches). A series of outpocketings in the neck region of an embryo, each of which consists of an epithelial pocket of endoderm and ectoderm that becomes filled by both mesoderm and cranial neural crest-derived mesenchymal cells. The first branchial arch gives rise to the jaws and other head structures.

METAMERIC
A form of segmentation by which all segments show an underlying serial homology.

FLOOR PLATE
The ventral-most longitudinal subdivision of the neural tube of the midbrain and the spinal cord, which acts as a local signalling centre.

VENTRICULAR ZONE
(Also called the proliferative zone). The part of the neuroepithelium that faces the ventricular (inner) surface of the neural tube, where cells are proliferating.

MANTLE ZONE
An outer layer of the neuroepithelium containing postmitotic neurons that have migrated radially away from the ventricular zone.

NEUROMERIC ORGANIZATION
The segmental organization of the neuroepithelium.

LUNATIC FRINGE
A glycosyl transferase that activates the Notch receptor and mediates differential sensitivity to various Notch ligands.

ALLOMETRIC GROWTH
Growth rates of a tissue vary along different axes in space, which drives shape changes of organs during embryogenesis.

AMNIOTE
Birds, reptiles and mammals are all amniotes; that is, their embryos are enclosed within an extraembryonic membrane, the amnion, which contains amniotic fluid. This provides a 'private pond' for the developing embryos of these land-dwelling vertebrates.

The ZLI is not a singular boundary but a compartment in its own right that is delimited by cell lineage restriction boundaries, both anteriorly and posteriorly[35]. At earlier stages, these two boundaries flank a wedge-shaped region that encompasses about one-third of the entire forebrain anlage and is characterized by a gap in the expression of LUNATIC FRINGE (*Lfng*). Subsequently, the *Lfng*-free wedge becomes progressively narrower with respect to the rest of the developing forebrain until it forms the narrow band of cells that constitutes the definitive ZLI[41] (FIGS 3,4). The reasons for this striking ALLOMETRIC GROWTH remain obscure.

The midbrain–hindbrain boundary

An important function of compartment boundaries in insect embryos is the stabilization of local signalling centres that direct the development of adjacent tissues. The boundary between the midbrain and the hindbrain (MHB), also known as the isthmus, has served as a model for a local signalling centre in the developing brain that is essential for the emergence of the midbrain and the cerebellum (anterior hindbrain; FIG. 1)[42–44]. Although the signalling function of the MHB has been the subject of intense investigation for some 15 years (see below), cell lineage restriction in this area has been controversial[43]. Fate-mapping experiments in AMNIOTE embryos have yielded conflicting results, with some finding[45,46] and others failing to find[47] the presence of a cell-tight boundary at the MHB.

Recent studies of quail–chick chimaeras have further complicated the issue by showing that isthmus cells themselves might contribute to dorsal parts of the midbrain and hindbrain[48,49]. A study that used an inducible transgenic marker in mice indicated

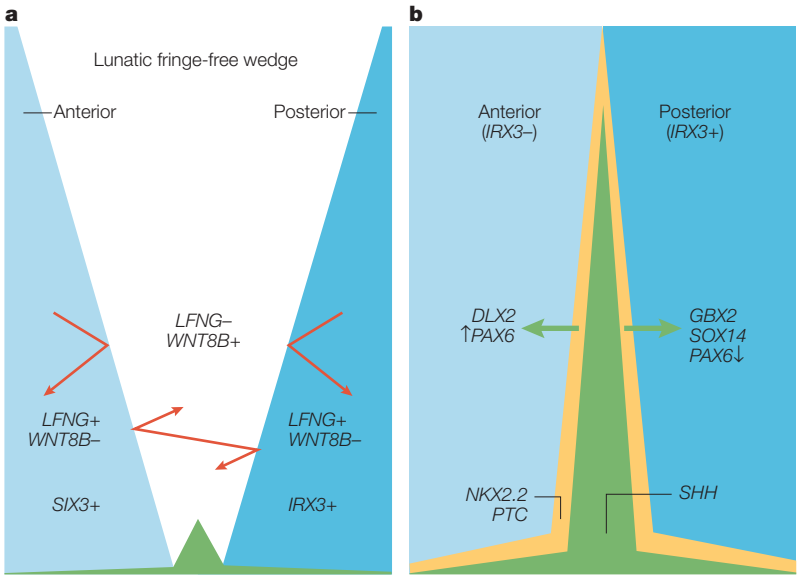

Figure 4 | **Lineage restriction at and signalling from the chick zona limitans intrathalamica. a** | The zona limitans intrathalamica (ZLI) forms from a lunatic fringe (*LFNG*)-negative, *WNT8B*-expressing wedge-shaped area that encompasses about one-third of the forebrain anlage. Anterior and posterior borders of this wedge function as cell lineage restriction boundaries (red arrows). The expression of *SIX3* (sine oculis homeobox 3) anterior to and *IRX3* (iroquois homeobox 3) posterior to this area indicates the presence of a prepattern. **b** | The definitive ZLI expresses sonic hedgehog (*SHH*), which is required for the induction of *DLX2* (distalless homeobox 2) in the prethalamus anteriorly and of *GBX2* (gastrulation brain homeobox 2) and *SOX14* (high mobility group (HMG) box transcription factor 14) in the thalamus posteriorly, as well as for the maintenance of *PAX6* (paired box gene 6) expression in the prethalamus and its downregulation in the thalamus. Other SHH target genes, such as patched (*PTC*) and *NKX2.2* (NK2 transcription factor related, locus 2), are induced on both sides of the ZLI (yellow).

the presence of cell lineage restriction between the midbrain and the isthmic area, and between the dorsal isthmus and the forming cerebellum (dorsal r1)[39]. Therefore, the isthmus might be a compartment rather than a single boundary, at least in dorsal aspects of the neural tube, similar to that described for the ZLI. The dorsal part of the midbrain–isthmus boundary seems to allow a minority of labelled cells to cross. Recently, an elegant time-lapse study that mapped the fates of hundreds of cells in the developing zebrafish MHB region clearly showed a cell lineage restriction boundary between the midbrain and r1 (REF. 50). Collectively, there is evidence for restricted cell movement at the MHB, and the isthmus might even form a separate compartment, but lineage restriction could be leaky under certain conditions in the chick and the mouse[39,47].

Boundaries as signalling centres

For a long time, the MHB was the only known example of a boundary that serves as a local signalling centre in the developing CNS[42–44]. Its inductive properties were first described for the chick, in which grafts of the isthmic region into other parts of the neural tube result in the ectopic induction of midbrain and cerebellum, an effect that can be mimicked by implanting beads soaked with fibroblast growth factor 8 (FGF8), the principal signalling molecule secreted by the MHB[51]. The MHB is able to induce cellular fates in a non-autonomous manner, like the ORGANIZER of the GASTRULA of vertebrate embryos, so the term 'secondary organizer' has been used for such signalling centres[52]. A requirement for FGF8 in midbrain and hindbrain development has been confirmed in mice[53] and in the zebrafish *fgf8* mutant *acerebellar*[54]. The *pou2* gene, which encodes a homeobox transcription factor of the Pou family and is disrupted in the zebrafish *spiel-ohne-grenzen* mutant, mediates the COMPETENCE of presumptive midbrain and hindbrain to respond to FGF signalling[55]. Initially, another signalling factor, Wnt1, is broadly expressed throughout the midbrain, until its expression becomes restricted to the dorsal midline and a narrow stripe anterior to the MHB, abutting the expression domain of *fgf8*. *Wnt1* mutant mice show severe midbrain deficits, yet ectopic application of WNT1 does not elicit inductive effects comparable to those of FGFs, which suggests a permissive role for WNT signalling in MHB function[42,43].

How can a single signal such as FGF elicit two fundamentally different responses on either side of its source — midbrain development anteriorly and cerebellum formation posteriorly? On the basis of misexpression experiments in chick embryos, it has been suggested that different FGFs secreted by the MHB differ in their biological activities. According to these studies, only FGF8B is able to induce cerebellar identity, whereas FGF8A, FGF17B and FGF18 only promote midbrain development without being able to induce ectopic structures[56,57]. However, these studies neither showed a requirement for different FGF isoforms nor were they able to explain why different FGFs should act in a unidirectional fashion from the MHB, exclusively affecting tissues anterior or posterior to their source.

The MHB is located at the interface of the expression domains of the homeobox genes *Otx2* (which is expressed in presumptive forebrain and midbrain) and gastrulation brain homeobox 2 (*Gbx2*; anterior hindbrain), and these genes might confer differential competence to respond to MHB-derived signals to tissues on both sides of the MHB[56]. However, *Otx2* and *Gbx2* are also likely to be involved in defining neural subdivisions independently of the MHB, and have been implicated in MHB positioning (see below), complicating the interpretation of gain- and loss-of-function approaches. *IRX2*, a homologue of the *Iroquois* family of homeobox genes that are involved in establishing prepatterning in *D. melanogaster*, is expressed in the presumptive anterior hindbrain before the onset of *FGF8* expression and has been shown to mediate the competence of this region to form the cerebellum in response to FGF signalling[58]. FGF8 is required to convert IRX2 from a transcriptional repressor into an activator: therefore, an activated form of IRX2 can convert presumptive tectum into cerebellum when misexpressed in the midbrain, whereas a repressor form of IRX2 has the opposite effect when electroporated into the hindbrain[58].

ORGANIZER
A small group of cells at the gastrula stage of vertebrate embryos that can induce a secondary embryonic axis in a non-autonomous fashion when transplantated into a host embryo.

GASTRULA
Early embryonic stage during which the just-formed germ layers are reorganized by extensive tissue movements.

COMPETENCE
The ability of a tissue to respond to an inducing signal.

Signalling functions have been revealed for other neuroepithelial boundaries. Several signalling factors of the Wnt family are expressed in zebrafish rhombomere boundaries, and three studies that used MORPHOLINO ANTISENSE OLIGONUCLEOTIDES against these Wnts or against the Wnt transducer Tcf3b revealed that Wnt signalling from rhombomere boundaries is required for maintaining rhombomere boundaries and patterned neurogenesis within rhombomeres[59–61]. However, Wnt expression at early rhombomere boundaries has not been described in other vertebrates.

Time-lapse studies have shown that r4 is the first rhombomere to form in zebrafish[62], as it is in other vertebrates. r4 is distinct from other rhombomeres because it expresses Fgf3 and Fgf8, and the release of these factors from r4 has been shown to be involved in local patterning, segmentation and neurogenesis in the hindbrain[62,63]. Therefore, r4 provides another example of a local signalling centre that is flanked both anteriorly and posteriorly by compartment boundaries.

The formation of the definitive ZLI in the forebrain is characterized by the expression of the signalling factor sonic hedgehog (SHH). For a considerable period during development, the ZLI is the only region of the neural tube where SHH (which is expressed along the length of the ventral midline from an early stage) protrudes dorsally, thereby forming a distinctive peak[41] (FIG. 3). The early developmental defects elicited by genetic disruption of *Shh* in mice have precluded the characterization of a ZLI-specific role for this signal[64,65]. We recently used an *in ovo* ELECTROPORATION approach in chicks to modulate SHH signalling in a spatiotemporally defined manner, and found that the ZLI functions as a local signalling centre that is essential for the establishment of its flanking regions — the prethalamus anteriorly and the thalamus posteriorly.

The *IRX2*-related gene *IRX3* is expressed exclusively posteriorly to the ZLI[66], and its ectopic misexpression anteriorly endows the prethalamus with thalamus-specific gene expression in a SHH-dependent manner[67], which indicates that a prepattern of IRX transcription factor expression regulates differential competence on either side of the signalling centre, as has already been shown for the MHB[58] (FIGS 3,4). The expression of genes that encode signalling factors other than SHH converges at the ZLI: *Fgf8* and *Fgf15* are expressed in the dorsal diencephalon[52,65], *Wnt3* and *Wnt3a* flank the ZLI posteriorly[68] and *Wnt8b* is expressed dorsally and in the ZLI itself[69] (FIG. 3). This raises the exciting possibility that the ZLI acts as a compound signalling centre that regulates the development of the posterior forebrain through the interaction of various pathways, but the roles of FGF and WNT signalling at these later stages of forebrain development remain to be established.

The anterior border of the neural plate (the 'anterior neural ridge' in mice and 'row-1' in zebrafish) also functions as a local signalling centre[70], whereby the region is crucially involved in forebrain patterning through its secretion of WNT antagonists during gastrulation[71] and of FGFs at later stages[72,73]. Whether this cell population, which gives rise to ventral parts of the telencephalon, the nasal pits and the PITUITARY GLAND, is located at a cell lineage restriction boundary has yet to be addressed.

Dorsoventral patterning of the neural tube is governed by two structures — the floor plate, which extends along the ventral midline of the neural tube and acts by emitting ventralizing signals such as SHH and Nodal[74], and the roof plate at the dorsal midline, which acts by secreting bone morphogenetic proteins and WNTs[75]. Although the signalling roles of the floor and roof plates have been subject to intense investigation and are relatively well understood, cell lineage restriction has not been exhaustively addressed in these areas. Fate-mapping experiments in the chick hindbrain have indicated that lineage restriction is present at the ventral midline, but not between the hindbrain floor plate and more dorsal parts of the neuroepithelium, or along its anteroposterior axis[10].

Signalling functions have not yet been described for the PSB, but several epidermal growth factor family members, FGF7 and the secreted WNT antagonist SFRP2 (secreted Frizzled-related protein 2) are expressed along this boundary, which indicates that it might constitute yet another local signalling centre[76,77]. Several *Wnt* genes are expressed in the dorsal midline of the developing telencephalon[78], and it is tempting to speculate that the PSB, through its expression of SFRP2, functions as a sink that is involved in shaping a gradient of WNT signalling along the dorsoventral axis of the emerging neocortex. How is it that the telencephalon is apparently the only subdivision of the neural tube to possess a dorsoventral lineage restriction boundary? It is the part of the developing brain that shows the highest complexity along its dorsoventral axis, and the emergence of a lateral signalling centre might be necessary to establish and refine this complex subregionalization.

A defining characteristic of an 'organizer' is its ability to induce ectopic cell fates in host tissue following heterotopic transplantation. This capability has been shown for the MHB, which can induce ectopic tectal and cerebellar structures on transplantation into the forebrain and hindbrain, respectively[42–44], and for the anterior neural boundary (ANB), which can induce anterior neural markers in the posterior neural plate[70], but not for the ZLI or for rhombomeres or their boundaries. Although grafting experiments have shown an organizer-like function of the MHB, they have also revealed that the competence to respond to MHB signalling is restricted within the neural tube: ectopic inductions of tectum or cerebellum in the forebrain are only observed posterior, but not anterior, to the ZLI, which indicates that the ZLI marks an important interface between regions of different competence[66].

Taken together, signalling functions have been ascribed to all of the characterized lineage restriction boundaries in the developing brain except for the DMB and the PSB. The signals secreted by these

MORPHOLINO ANTISENSE OLIGONUCLEOTIDES
Synthetic oligonucleotides that are exceptionally stable and can serve as tools to block translation or RNA splicing.

ELECTROPORATION
A technique for gene delivery into cells, which allows the transfer of expression plasmids or morpholinos to groups of cells in living embryos.

PITUITARY GLAND
An endocrine gland that forms through an interaction between neuroectoderm and oral ectoderm.

boundaries might refine local tissue patterning in a MORPHOGEN-like fashion or act on prepatterned tissue to regulate the temporal progression of gene expression, or both. However, there is no strict requirement for lineage restriction in the establishment of local signalling centres. We can hypothesize that a signalling centre that is not stabilized by lineage restriction consists of cells of labile fate that must continue to be able to 'sense' their position within the embryo, for example, in relation to global patterning gradients.

Positioning of boundaries

Given their importance as local organizers of neural development, it is of considerable interest to understand how boundaries are positioned in the emerging CNS. This process is best understood for the MHB, which forms where the expression domains of the homeobox genes *Otx2* and *Gbx2* (*gbx1* in zebrafish) abut. Regionalized expression of these two genes is first detected in the neural plate during gastrulation, which indicates that MHB positioning is governed by the same mechanisms that regulate the earliest steps of anteroposterior neural patterning. Anterior neural tissue is progressively posteriorized by signalling gradients in the gastrula stage embryo, and FGFs, Nodals, retinoic acid and WNTs have been suggested to be involved in this process[79,80]. Recently, WNTs have emerged as particularly good candidates for this role: WNT signalling represses *Otx2* and induces *Gbx2* (*gbx1*) directly in neural tissue, without a MESODERMAL intermediate[59,81–83]. So, a direct line can be drawn from early neural patterning, which is mediated by global gradients, to the subsequent refinement of this crude pattern through the activity of a local signalling centre, the MHB, which is induced at a specific anteroposterior position as a read-out of the gradients.

A similar mechanism of induction has been proposed for the ZLI, which seems to form at the interface between a *SIX3*-expressing territory anteriorly and an *IRX3*-expressing territory posteriorly[66,68]. Like *OTX2*, *SIX3* is repressed by canonical WNT signalling[68,84] and, like *GBX2*, *IRX3* is induced by WNTs[68], which indicates that ZLI formation occurs at a specific threshold of WNT activity in the gastrulating embryo[85]. However, experimental evidence that ZLI formation is established at a *SIX3*/*IRX3* expression border is lacking. Furthermore, how the expression domains of *SIX3* and *IRX3* relate to the *LFNG*-free territory that constitutes the presumptive ZLI has yet to be resolved[41]. It has recently been proposed that ZLI formation is promoted by ventral SHH signalling and antagonized by unidentified signals from the dorsal diencephalon[86]. So, it seems that extracellular signals generate a Cartesian coordinate system whereby the ZLI emerges at specific axial positions.

Rhombomere boundaries are impaired following experimental abrogation of the expression of *Hox* genes, HOX cofactors and other rhombomere marker genes such as *Krox20* (REFS 87–90). Therefore, *Hox* function is likely to have a dual role during hindbrain development, both determining segmental identities and regulating segmentation itself. Distinguishing between these two functions might prove difficult, as *Hox* genes and their cofactors show extensive cross-regulation. Several lines of evidence show that *Hox* gene expression is under the global control of retinoic acid signalling, and it has been proposed that a gradient of retinoic acid signalling and/or response (low anteriorly, high posteriorly) controls the hierarchy of hindbrain gene expression[91,92]. Therefore, hindbrain segmentation might be another example of the translation of an early gradient that induces a crude anteroposterior pattern into a refined domain structure with a high degree of local patterning.

Interfering with SHH signalling from the ZLI results in the loss of its specific gene expression profile (including loss of the expression of *Shh* itself)[67,86]. Similarly, zebrafish that lack the Fgf competence factor Pou2 show disrupted MHB development[55], and the morphological constriction of the isthmus does not form in mice that are deficient in FGF receptor 1 at the MHB owing to a failure to downregulate proliferation in the boundary region[93]. Moreover, in the zebrafish hindbrain, Wnt signalling is necessary for the maintenance of defined boundaries[59–61]. These findings indicate a common theme in which, once a local signalling centre has been established along a boundary, its maintenance becomes dependent on the secreted signal itself. A similar phenomenon has been observed at anteroposterior compartment boundaries in the *D. melanogaster* wing IMAGINAL DISC and abdomen, the integrity of which depends on the activity of the morphogen hedgehog[94–96]. However, it is important to keep in mind that different boundary properties were analysed in the studies mentioned above. It is quite possible that different aspects of boundary formation — such as morphological changes and the expression of boundary marker genes — are regulated differently and that one persists in the absence of the other.

The DMB forms at the interface between the expression domains of the *Pax6* and engrailed (*En*) genes. Ectopic expression of *Pax6* posteriorly or of *En* anteriorly shifts this boundary to the new expression interface[97–99]. Loss-of-function experiments in zebrafish indicate that *en* and Fgf signalling from the MHB are required together to localize the DMB[99]. So, DMB positioning differs from the positioning of the other boundaries discussed above, as it depends not only on an anteroposterior prepattern in the early neural plate, but also on the activity of a secondary signalling centre, the MHB. *En* is also involved in boundary formation in *D. melanogaster*, in which it is required for the establishment of the posterior compartment of the developing wing[3], presumably through the control of *hedgehog* expression[94,95] (FIG. 5a).

Mechanisms of boundary formation

Historically, different mechanisms were proposed for the establishment of cell lineage restriction, either between compartments or between germ layers[100]. In an extension of ideas presented by Holtfreter[101],

MORPHOGEN
A secreted factor that can induce more than two different cell fates over a sheet of cells in a concentration-dependent manner by forming a gradient.

MESODERM
Germ layer that forms in between ectoderm and endoderm. Mesoderm is crucially involved in neural patterning during gastrulation.

IMAGINAL DISCS
Epithelial pouches in insect larvae that give rise to the sensory organs and body appendages of the adult.

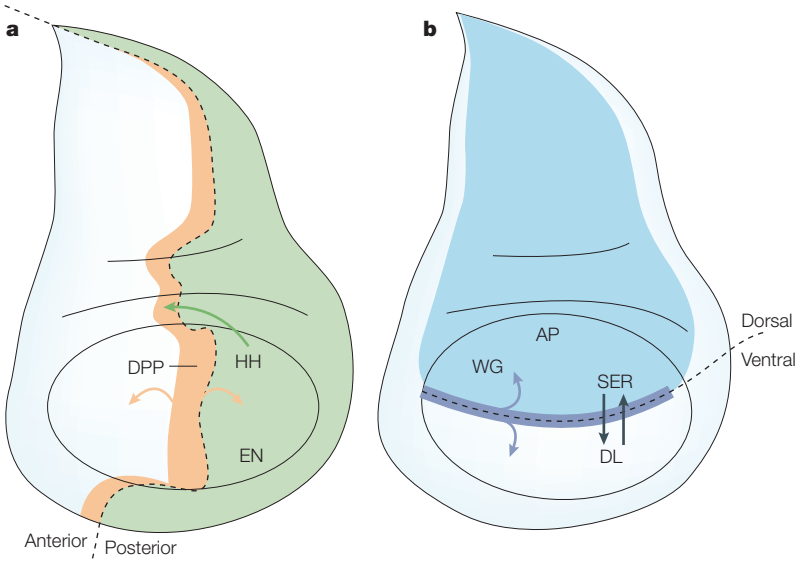

Figure 5 | **Compartment boundaries and local signalling centres in the developing fly wing. a** | The wing anlage in *Drosophila melanogaster* is subdivided anteroposteriorly by a cell lineage restriction boundary that expresses the morphogen Decapentaplegic (DPP, orange). The posterior compartment expresses Hedgehog (HH, green), the expression of which depends on Engrailed (EN). **b** | The dorsal compartment of the wing disc expresses the Notch modulator Fringe (pale blue) under the control of the transcription factor Apterous (AP), which results in activation of the Notch ligands Delta (DL) and Serrate (SER) at the dorsoventral compartment boundary (dark blue). Once the boundary has been established, it expresses the morphogen Wingless (WG), which regulates patterning of the wing margin and outgrowth of the wing blade. Panels **a** and **b** reproduced, with permission, from REF. 6 © (1999) Elsevier Science.

Steinberg and colleagues developed the differential adhesion hypothesis in the 1960s to explain the observation that dissociated embryonic cells tend to segregate and form clusters that represent their layer of origin. They proposed that different adhesive properties of the cell surfaces underlie this phenomenon and that the sorting-out of cells is driven by thermodynamic principles similar to those governing the separation of two immiscible liquids[102]. Although this model was not initially proposed to account for the phenomenon of developmental compartments, it provides a ready explanation for how cells from adjacent regions might be prevented from intermingling. Alternatively, boundaries might act as mechanical barriers between populations of cells by generating specialized boundary cells or by increasing the deposition of extracellular matrix, either of which could act like a fence.

Rhombomere boundaries express specific boundary markers and are characterized by an enlarged intercellular space, the accumulation of RADIAL GLIA and extracellular matrix components, and the precocious formation of a marginal zone[13,18,19], any or all of which could function as a mechanical barrier. However, no intermixing between cells from adjacent rhombomeres is observed after surgical ablation of rhombomere boundaries[17] or if boundary cell formation is inhibited by treatment with retinoic acid[103]. These observations indicate that the formation of boundary cells is not the primary cause of cell lineage restriction between rhombomeres.

RADIAL GLIA
Glial cells that span the radial axis of neuroepithelium and serve as guidance cues for newly born postmitotic neurons on their way into the mantle zone.

In vitro experiments using dissociated cells have revealed differential affinities between even- and odd-numbered rhombomere populations: reaggregation of cells from two even- or two odd-numbered rhombomeres resulted in homogenously mixed aggregates, whereas even and odd cells sort out into discrete domains in aggregates derived from even and odd rhombomeres[104]. Therefore, differential adhesiveness between adjacent rhombomeres is a probable mechanism for the restriction of cell intermingling, whereas the subsequent formation of a specialized boundary at the interface of immiscibility might further stabilize the initial partitioning. The increase in extracellular space at early rhombomere boundaries[18] is also consistent with loss of adhesion between cells with different surface properties and their (incomplete) separation.

An analysis of cell movements at the PSB revealed that cells 'slow down' within the boundary region, which indicates that the secretion of a short-range signal that inhibits cell migration is one mechanism by which cell mixing is prevented at the PSB[105]. In addition, radial glia coalesce at the PSB as at rhombomere boundaries, which results in a specific boundary phenotype[105].

Recent studies have begun to shed light on the molecular mechanisms that underlie boundary formation. Members of the cadherin superfamily of cell adhesion molecules are expressed differentially in subdivisions of the brain, indicating their candidature as mediators of affinity differences between neuroepithelial compartments[106,107]. Gain-of-function experiments in mouse embryos have implicated the differential expression of two cadherins in the establishment of the PSB[108]. To thoroughly understand the role of cadherins in the mediation of compartition might prove exceedingly difficult, as not only the qualitative molecular differences but also expression levels of cadherins are likely to influence the adhesive properties of a cell[109].

Signalling through ephrin receptors (Eph) is known to regulate contact-mediated repulsion in both the nervous and the vascular systems[110,111]. Various Eph receptors are expressed in odd-numbered rhombomeres, whereas their ligands — membrane-spanning proteins of the ephrin-B family — are expressed in a complementary fashion in even-numbered rhombomeres. Experiments in zebrafish have shown that ectopic activation of Eph receptors in even-numbered rhombomeres, as well as ectopic ephrin activation, can cause expressing cells to sort out towards rhombomere boundaries[112]. Furthermore, it has also been shown that this segregation behaviour relies on bidirectional signalling, with both the Eph receptors and the ephrins transducing an intracellular signal.

Moreover, unidirectional Eph–ephrin interactions regulate intercellular communication through gap junctions[113]. Conversely, blocking Eph signalling by morpholino knockdown or by using a dominant-negative version of EphA4 results in disruption of hindbrain segmentation[20,114]. These findings indicate

Table 1 | **Boundaries in the developing vertebrate brain**

Regional interface	Cell lineage restriction	Signalling function
Anterior neural border (ANB)	?	+ (anti-WNT, FGFs)
Pallial–subpallial boundary (PSB)	+ (Ventricular zone only)	None detected
Telencephalon–diencephalon	–	None detected
Zona limitans intrathalamica (ZLI)	+ (Two boundaries with lineage restriction anteriorly and posteriorly; does not extend into roof plate)	+ (SHH, WNTs?, FGFs?)
Thalamus–pretectum	–	None detected
Diencephalic–midbrain boundary (DMB)	+	None detected
Midbrain–hindbrain boundary (MHB)	+ (Might be leaky; possibly two boundaries dorsally)	+ (FGFs, WNT1)
Rhombomeres	+ (Except floor plate; ventricular zone only)	+ (WNT1, WNT3A?, WNT8B?, WNT10B?)
Spinal cord	–	Anteroposterior: – Dorsoventral: +

FGF, fibroblast growth factor; SHH, sonic hedgehog.

a model for partitioning the hindbrain whereby complementarily expressed Eph receptors and ephrins make contact only at presumptive rhombomere boundaries, which results in repulsion between cells of adjacent rhombomeres and the consequent formation of lineage-restricted compartments. In addition, Eph–ephrin signalling also seems to influence intra-rhombomeric cell affinities, as was recently revealed using a mosaic knockdown of EphA4 in zebrafish[114]. Notably, OTX2 seems to regulate R-cadherin and ephrinA2 in mice, which indicates a link between early prepatterning and the later establishment of differential cellular adhesiveness around the MHB[115].

NOTCH is another signalling factor that mediates communication between neighbouring cells or populations of cells — for example, in lateral inhibition in various embryonic tissues and in neurogenesis[116]. *Radical fringe* (*rfng*), a putative regulator of Notch, is expressed in rhombomere boundary cells in zebrafish, and expression of *delta*, which encodes a Notch ligand, straddles the boundaries. Mosaic expression of a Notch pathway activator in zebrafish embryos results in cells with hyperactive Notch signalling segregating to boundaries, whereas, conversely, cells in which the Notch pathway is inhibited become excluded from boundaries[21]. The expression of *wnt1* in rhombomere boundaries of the fish is essential for this Notch-mediated segmentation, which indicates a similarity with dorsoventral boundary formation in the *D. melanogaster* wing anlage[60,61] (FIG. 5b). Notably, Notch activation has also been implicated in compartition in the vertebrate forebrain: the wedge-shaped area that gives rise to the ZLI is characterized by a gap in the expression of *LFNG*, another potential regulator of Notch signalling. Ectopic expression of *LFNG* in the pre-ZLI compartment results in sorting of the affected cells into the *LFNG*-positive flanking regions[41].

Together, both differential adhesion and the establishment of specialized boundary features seem to synergize in the formation of cell lineage restriction boundaries. Although differential adhesion might be mediated by a broad combination of different cell adhesion molecules, boundary cells are stabilized by positive feedback loops that involve Notch signalling. In addition, Eph–ephrin-mediated repulsive interactions seem to restrict intermingling between neighbouring compartments. Boundary cells are not strictly required for lineage restriction and the initial formation of neural compartments, and they regenerate quickly after ablation. This indicates that the establishment of adhesive differences constitutes the first step of lineage restriction, whereas fence-type mechanisms might stabilize compartments at later stages.

Conclusions and future directions

Boundary formation and the activity of local signalling centres are key features of vertebrate brain development. Although a good case has been made for the hindbrain forming in a segmented fashion, there is little evidence for a neuromeric organization of the forebrain. In this area, the emerging diencephalic subregions have individual molecular profiles but lack the shared, reiterated features that characterize a segmental ground plan. It is tempting to speculate that the phylogenetically younger forebrain shows greater morphological variability between different species because it is less restricted by a compartmental organization.

The ongoing debate about lineage restriction in different parts of the brain probably reflects differences in experimental approaches and highlights the danger of defining compartments solely on the basis of gene expression data. Different lineage-tracing techniques are likely to yield varying results as to where boundaries are present. Orthotopic grafting of quail tissue into chick embryos is a classic approach for mapping cell fates in avian embryos[28,45,48,49,69]; however, its resolution is limited by the size of the grafts and it bears the inherent danger that cellular behaviours might be changed as a result of the wounding of embryonic tissue and the subsequent integration of the graft. Application of lipophilic dyes such as DiI or DiO is a less invasive method of generating labelled cells for which resolution is mainly limited by how focally the label can be applied[27,29,30,46,105,108]. Smaller groups or even single cells can be labelled iontophoretically with conjugated dextrans[10,27,29,35,47].

All of these techniques are of limited use in the mouse embryo, which can be kept in culture for only a short developmental period[30,46,108]. Cells in the developing mouse neural tube can be marked *in utero*

NOTCH
A receptor at the heart of a signalling pathway that regulates a multitude of developmental decisions.

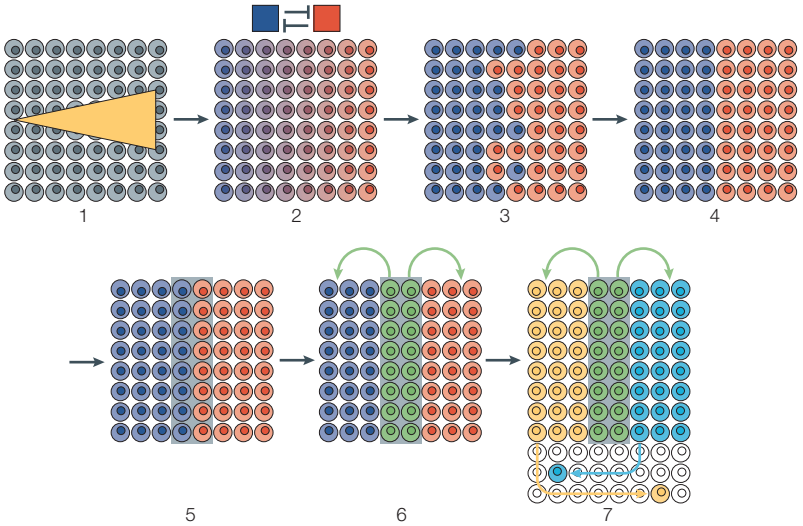

Figure 6 | **Model for boundary formation.** An initially uniform sheet of cells is polarized by an early signalling gradient (yellow; 1), which results in a coarse prepattern of transcription factor expression (red/blue; 2). Mutual repressive interactions between these factors establish two distinct populations of cells that are separated by a fuzzy interface (3). Cell-sorting processes result in a sharpening of this interface (4), and a specific boundary phenotype (loss of adhesion, expression of specific boundary markers) is generated (shaded area; 5). The boundary cells express signalling factors (green; 6) that induce prepattern-dependent cell fates (yellow/turquoise) in the adjacent territories. Postmitotic cells might be able to cross the boundary, as their fates are sealed (7).

using replication-incompetent retroviruses that contain a reporter gene[36] or by intragenic homologous recombination[117], but the labelling occurs in a random fashion in both approaches and the exact time point of reporter activation cannot be determined retrospectively. The genetic labelling of groups of cells using a tissue-specific marker that drives a reporter gene reflects patterns of gene expression rather than cellular behaviour, and cannot exclude the possibility of boundaries being overlooked. Recently, more sophisticated experimental tools, such as inducible transgenic markers, have come into use and will allow the further identification and characterization of cell lineage restriction boundaries during vertebrate neural development[39]. Furthermore, imaging the fate of all cells of a given embryonic region by time-lapse microscopy is now feasible, but, for the time being, remains limited to the transparent zebrafish embryo, which has a relatively small number of cells and can easily be kept in culture[50,118].

With the exception of the DMB and the PSB, signalling functions have been attributed to all neuroepithelial cell lineage restriction boundaries (TABLE 1), which indicates that one of their main functions is the formation of local signalling centres, as in insect development (FIG. 6). The activity of these signalling centres (sometimes referred to as 'secondary organizers') fine-tunes spatiotemporal patterning, proliferation and morphogenesis of the neural tube in a more local fashion. However, a crude neural pattern has already been established before local signalling centres become active. The presence of an underlying prepattern not only regulates the positioning of boundaries, but also influences the way that flanking cell populations respond differently to a common diffusible signal[55,56,58,66,67]. Understanding this cellular competence is only in its infancy, but modern approaches that enable us to monitor the entire transcriptional profile of a tissue will soon bring inherent differences between cell populations to light.

Determining the cellular mechanisms of lineage restriction is an area of ongoing research[100]. As for the detection of boundaries, different experimental approaches might result in different perceptions of how cell populations segregate. The observation that cells from different neuroepithelial compartments or with different molecular properties are able to sort out *in vitro*[104,109] and *in vivo*[21,41,112–114,119] supports the idea that differential cell affinities underlie compartition. However, the mechanism by which a cell that is located in the 'wrong' compartment can actively move across the distance of many cell diameters to end up in the 'right' compartment remains an open question. It is important to keep in mind that experiments that address cell sorting typically create artificial situations that do not reflect the process of compartition in an embryo, in which cells are not initially intermixed and are unlikely to become misallocated to the wrong environment. In other words, active cell sorting might not be necessary to generate embryonic compartments, for which the processes involved are preventative of mixing rather than corrective.

Finally, how lineage restriction boundaries are established molecularly remains largely unexplored. The implication of a network of Wnt and Notch signalling in rhombomere boundary formation in zebrafish raises the exciting possibility that a SIGNALLING MODULE conserved between *D. melanogaster* and vertebrates functions in boundary formation (FIG. 5). It will be of considerable interest to identify further similarities between these two systems and to characterize vertebrate counterparts of factors that are well-characterized in *D. melanogaster* boundary formation.

Furthermore, Notch signalling has been implicated in SOMITOGENESIS in vertebrates[120,121]. Mesodermal segmentation is markedly different from neuroepithelial segmentation, as it involves the budding of somites from a proliferating growth zone rather than the internal subdivision of a preformed tissue mass. However, it is conspicuous that two sets of molecular regulators seem to be conserved between the two: first, the Notch network that is reiteratively activated at both somite and rhombomere boundaries; and, second, the nested expression of *Hox* genes that regulates anteroposterior segmental identity. Similarly, regulators of segmentation are largely conserved between LONG-GERM insects such as *D. melanogaster* and SHORT-GERM insects such as the beetle *Tribolium castaneum*, although in *T. castaneum* — unlike in *D. melanogaster* — segments form in an anteroposterior sequence from a proliferative zone[122]. So, hindbrain and mesoderm segmentation might be more similar than previously thought, despite their morphogenetic differences.

SIGNALLING MODULE
A group of signalling molecules of more than just one pathway that is reiteratively used in different tissues.

SOMITOGENESIS
Segmentation of paraxial mesoderm, which results in the formation of two stripes of distinctive mesodermal blocks along the anteroposterior axis that will give rise to muscle, vertebrae and dermis.

LONG- AND SHORT-GERM DEVELOPMENT
Different modes of insect development; in long-germ insects, all segments are formed from the blastoderm, whereas in short-germ insects, segments are formed by sequential growth.

1. Garcia-Bellido, A., Ripoll, P. & Morata, G. Developmental compartmentalisation of the wing disk of *Drosophila*. *Nature New Biol.* **245**, 251–253 (1973).
2. Lawrence, P. A. A clonal analysis of segment development in *Oncopeltus* (Hemiptera). *J. Embryol. Exp. Morphol.* **30**, 681–699 (1973).
3. Morata, G. & Lawrence, P. A. Control of compartment development by the *engrailed* gene in *Drosophila*. *Nature* **255**, 614–617 (1975).
4. Lawrence, P. A. & Struhl, G. Morphogens, compartments, and pattern: lessons from *Drosophila*? *Cell* **85**, 951–961 (1996).
5. Vincent, J. P. Compartment boundaries: where, why and how? *Int. J. Dev. Biol.* **42**, 311–315 (1998).
6. Dahmann, C. & Basler, K. Compartment boundaries: at the edge of development. *Trends Genet.* **15**, 320–326 (1999).
7. Mann, R. S. & Morata, G. The developmental and molecular biology of genes that subdivide the body of *Drosophila*. *Annu. Rev. Cell Dev. Biol.* **16**, 243–271 (2000).
8. Irvine, K. D. & Rauskolb, C. Boundaries in development: formation and function. *Annu. Rev. Cell Dev. Biol.* **17**, 189–214 (2001).
9. Vaage, S. The segmentation of the primitive neural tube in chick embryos (*Gallus domesticus*). *Ergebnisse der Anatomie und Entwicklungsgeschichte* **41**, 3–87 (1969).
10. Fraser, S., Keynes, R. & Lumsden, A. Segmentation in the chick embryo hindbrain is defined by cell lineage restrictions. *Nature* **344**, 431–435 (1990).
The first study to reveal cell lineage restriction at rhombomere boundaries of the vertebrate hindbrain.
11. Wilkinson, D. G., Bhatt, S., Cook, M., Boncinelli, E. & Krumlauf, R. Segmental expression of *Hox-2* homoeobox-containing genes in the developing mouse hindbrain. *Nature* **341**, 405–409 (1989).
The first report of segmental gene expression in the vertebrate hindbrain.
12. Hanneman, E., Trevarrow, B., Metcalfe, W. K., Kimmel, C. B. & Westerfield, M. Segmental pattern of development of the hindbrain and spinal cord of the zebrafish embryo. *Development* **103**, 49–58 (1988).
13. Lumsden, A. & Keynes, R. Segmental patterns of neuronal development in the chick hindbrain. *Nature* **337**, 424–428 (1989).
14. Trevarrow, B., Marks, D. L. & Kimmel, C. B. Organization of hindbrain segments in the zebrafish embryo. *Neuron* **4**, 669–679 (1990).
15. Clarke, J. D. & Lumsden, A. Segmental repetition of neuronal phenotype sets in the chick embryo hindbrain. *Development* **118**, 151–162 (1993).
16. Eickholt, B. J., Graham, A., Lumsden, A. & Wizenmann, A. Rhombomere interactions control the segmental differentiation of hindbrain neurons. *Mol. Cell. Neurosci.* **18**, 141–148 (2001).
17. Guthrie, S. & Lumsden, A. Formation and regeneration of rhombomere boundaries in the developing chick hindbrain. *Development* **112**, 221–229 (1991).
18. Heyman, I., Kent, A. & Lumsden, A. Cellular morphology and extracellular space at rhombomere boundaries in the chick embryo hindbrain. *Dev. Dyn.* **198**, 241–253 (1993).
19. Heyman, I., Faissner, A. & Lumsden, A. Cell and matrix specialisations of rhombomere boundaries. *Dev. Dyn.* **204**, 301–315 (1995).
20. Xu, Q., Alldus, G., Holder, N. & Wilkinson, D. G. Expression of truncated Sek-1 receptor tyrosine kinase disrupts the segmental restriction of gene expression in the *Xenopus* and zebrafish hindbrain. *Development* **121**, 4005–4016 (1995).
21. Cheng, Y. C. *et al.* Notch activation regulates the segregation and differentiation of rhombomere boundary cells in the zebrafish hindbrain. *Dev. Cell* **6**, 539–550 (2004).
Provides evidence that activation of the Notch pathway *in vivo* directs cells to rhombomere boundaries in zebrafish.
22. Studer, M., Lumsden, A., Ariza-McNaughton, L., Bradley, A. & Krumlauf, R. Altered segmental identity and abnormal migration of motor neurons in mice lacking *Hoxb-1*. *Nature* **384**, 630–634 (1996).
Shows that expression of a singular *Hox* gene, *Hoxb1*, is required to define r4-specific characteristics.
23. McClintock, J. M., Kheirbek, M. A. & Prince, V. E. Knockdown of duplicated zebrafish *hoxb1* genes reveals distinct roles in hindbrain patterning and a novel mechanism of duplicate gene retention. *Development* **129**, 2339–2354 (2002).
24. Bell, E., Wingate, R. J. & Lumsden, A. Homeotic transformation of rhombomere identity after localized *Hoxb1* misexpression. *Science* **284**, 2168–2171 (1999).
Complements reference 22 by showing that *Hoxb1* is sufficient to induce r4 character ectopically in r2. Taken together, the two studies indicate that *Hoxb1* functions as a selector gene for r4 identity.
25. Jungbluth, S., Bell, E. & Lumsden, A. Specification of distinct motor neuron identities by the singular activities of individual *Hox* genes. *Development* **126**, 2751–2758 (1999).
26. Awatramani, R., Soriano, P., Rodriguez, C., Mai, J. J. & Dymecki, S. M. Cryptic boundaries in roof plate and choroid plexus identified by intersectional gene activation. *Nature Genet.* **35**, 70–75 (2003).
27. Birgbauer, E. & Fraser, S. E. Violation of cell lineage restriction compartments in the chick hindbrain. *Development* **120**, 1347–1356 (1994).
28. Wingate, R. J. & Lumsden, A. Persistence of rhombomeric organisation in the postsegmental hindbrain. *Development* **122**, 2143–2152 (1996).
29. Figdor, M. C. & Stern, C. D. Segmental organization of embryonic diencephalon. *Nature* **363**, 630–634 (1993).
The first study that combines morphological considerations, gene expression data and fate mapping in an attempt to define a neuromeric organization of the diencephalon.
30. Fishell, G., Mason, C. A. & Hatten, M. E. Dispersion of neural progenitors within the germinal zones of the forebrain. *Nature* **362**, 636–638 (1993).
31. Simeone, A., Acampora, D., Gulisano, M., Stornaiuolo, A. & Boncinelli, E. Nested expression domains of four homeobox genes in developing rostral brain. *Nature* **358**, 687–690 (1992).
The first report of a nested expression of homeobox genes in the early forebrain.
32. Reichert, H. Conserved genetic mechanisms for embryonic brain patterning. *Int. J. Dev. Biol.* **46**, 81–87 (2002).
33. Rubenstein, J. L., Martinez, S., Shimamura, K. & Puelles, L. The embryonic vertebrate forebrain: the prosomeric model. *Science* **266**, 578–580 (1994).
The original version of the prosomeric model, which proposes that the developing forebrain is segmented and consists of six neuromeres.
34. Bell, E., Ensini, M., Gulisano, M. & Lumsden, A. Dynamic domains of gene expression in the early avian forebrain. *Dev. Biol.* **236**, 76–88 (2001).
35. Larsen, C. W., Zeltser, L. M. & Lumsden, A. Boundary formation and compartition in the avian diencephalon. *J. Neurosci.* **21**, 4699–4711 (2001).
36. Arnold-Aldea, S. A. & Cepko, C. L. Dispersion patterns of clonally related cells during development of the hypothalamus. *Dev. Biol.* **173**, 148–161 (1996).
37. Golden, J. A. & Cepko, C. L. Clones in the chick diencephalon contain multiple cell types and siblings are widely dispersed. *Development* **122**, 65–78 (1996).
38. Szele, F. G. & Cepko, C. L. The dispersion of clonally related cells in the developing chick telencephalon. *Dev. Biol.* **195**, 100–113 (1998).
39. Zervas, M., Millet, S., Ahn, S. & Joyner, A. L. Cell behaviors and genetic lineages of the mesencephalon and rhombomere 1. *Neuron* **43**, 345–357 (2004).
State-of-the-art study that used genetic fate mapping with an inducible transgene in mice, which revealed cell lineage restriction at the DMB and the MHB.
40. Puelles, L. & Rubenstein, J. L. Forebrain gene expression domains and the evolving prosomeric model. *Trends Neurosci.* **26**, 469–476 (2003).
The latest incarnation of the prosomeric model, which proposes that the diencephalon consists of three neuromeres, whereas the telencephalon is unsegmented.
41. Zeltser, L. M., Larsen, C. W. & Lumsden, A. A new developmental compartment in the forebrain regulated by *Lunatic fringe*. *Nature Neurosci.* **4**, 683–684 (2001).
42. Liu, A. & Joyner, A. L. Early anterior/posterior patterning of the midbrain and cerebellum. *Annu. Rev. Neurosci.* **24**, 869–896 (2001).
43. Wurst, W. & Bally-Cuif, L. Neural plate patterning: upstream and downstream of the isthmic organizer. *Nature Rev. Neurosci.* **2**, 99–108 (2001).
44. Raible, F. & Brand, M. Divide et Impera — the midbrain–hindbrain boundary and its organizer. *Trends Neurosci.* **27**, 727–734 (2004).
45. Millet, S., Bloch-Gallego, E., Simeone, A. & Alvarado-Mallart, R. M. The caudal limit of *Otx2* gene expression as a marker of the midbrain/hindbrain boundary: a study using *in situ* hybridisation and chick/quail homotopic grafts. *Development* **122**, 3785–3797 (1996).
46. Inoue, T., Nakamura, S. & Osumi, N. Fate mapping of the mouse prosencephalic neural plate. *Dev. Biol.* **219**, 373–383 (2000).
47. Jungbluth, S., Larsen, C., Wizenmann, A. & Lumsden, A. Cell mixing between the embryonic midbrain and hindbrain. *Curr. Biol.* **11**, 204–207 (2001).
48. Alexandre, P. & Wassef, M. The isthmic organizer links anteroposterior and dorsoventral patterning in the mid/hindbrain by generating roof plate structures. *Development* **130**, 5331–5338 (2003).
49. Louvi, A., Alexandre, P., Metin, C., Wurst, W. & Wassef, M. The isthmic neuroepithelium is essential for cerebellar midline fusion. *Development* **130**, 5319–5330 (2003).
50. Langenberg, T. & Brand, M. Neuromeric properties of the midbrain–hindbrain boundary region in zebrafish. *Development* (in the press).
This study exploits the advantages of the zebrafish embryo — transparency and the comparably small number of cells — for characterizing cell lineage restriction at the MHB.
51. Crossley, P. H., Martinez, S. & Martin, G. R. Midbrain development induced by FGF8 in the chick embryo. *Nature* **380**, 66–68 (1996).
Landmark study showing that the inducing activity of the MHB can be mimicked by FGF8.
52. Echevarria, D., Vieira, C., Gimeno, L. & Martinez, S. Neuroepithelial secondary organizers and cell fate specification in the developing brain. *Brain Res. Brain Res. Rev.* **43**, 179–191 (2003).
53. Chi, C. L., Martinez, S., Wurst, W. & Martin, G. R. The isthmic organizer signal FGF8 is required for cell survival in the prospective midbrain and cerebellum. *Development* **130**, 2633–2644 (2003).
54. Reifers, F. *et al. Fgf8* is mutated in zebrafish *acerebellar* (*ace*) mutants and is required for maintenance of midbrain–hindbrain boundary development and somitogenesis. *Development* **125**, 2381–2395 (1998).
55. Reim, G. & Brand, M. *spiel-ohne-grenzen/pou2* mediates regional competence to respond to Fgf8 during zebrafish early neural development. *Development* **129**, 917–933 (2002).
56. Sato, T., Araki, I. & Nakamura, H. Inductive signal and tissue responsiveness defining the tectum and the cerebellum. *Development* **128**, 2461–2469 (2001).
57. Liu, A. *et al.* FGF17b and FGF18 have different midbrain regulatory properties from FGF8b or activated FGF receptors. *Development* **130**, 6175–6185 (2003).
58. Matsumoto, K. *et al.* The prepattern transcription factor Irx2, a target of the FGF8/MAP kinase cascade, is involved in cerebellum formation. *Nature Neurosci.* **7**, 605–612 (2004).
59. Dorsky, R. I., Itoh, M., Moon, R. T. & Chitnis, A. Two *tcf3* genes cooperate to pattern the zebrafish brain. *Development* **130**, 1937–1947 (2003).
60. Riley, B. B. *et al.* Rhombomere boundaries are Wnt signaling centers that regulate metameric patterning in the zebrafish hindbrain. *Dev. Dyn.* **231**, 278–291 (2004).
61. Amoyel, M., Cheng, Y.-C., Jiang, Y.-J. & Wilkinson, D. G. Wnt regulates neurogenesis and mediates lateral inhibition of boundary cell specification in the zebrafish hindbrain. *Development* **132**, 775–785 (2005).
62. Maves, L., Jackman, W. & Kimmel, C. B. FGF3 and FGF8 mediate a rhombomere 4 signaling activity in the zebrafish hindbrain. *Development* **129**, 3825–3837 (2002).
63. Walshe, J., Maroon, H., McGonnell, I. M., Dickson, C. & Mason, I. Establishment of hindbrain segmental identity requires signaling by FGF3 and FGF8. *Curr. Biol.* **12**, 1117–1123 (2002).
64. Chiang, C. *et al.* Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature* **383**, 407–413 (1996).
65. Ishibashi, M. & McMahon, A. P. A sonic hedgehog-dependent signaling relay regulates growth of diencephalic and mesencephalic primordia in the early mouse embryo. *Development* **129**, 4807–4819 (2002).
66. Kobayashi, D. *et al.* Early subdivisions in the neural plate define distinct competence for inductive signals. *Development* **129**, 83–93 (2002).
Shows that the transcription factors SIX3 and IRX3 are sufficient to define anterior and posterior domains of competence to respond to signals from the anterior neural border, the MHB and the floor plate.
67. Kiecker, C. & Lumsden, A. Hedgehog signaling from the ZLI regulates diencephalic regional identity. *Nature Neurosci.* **7**, 1242–1249 (2004).
68. Braun, M. M., Etheridge, A., Bernard, A., Robertson, C. P. & Roelink, H. Wnt signaling is required at distinct stages of development for the induction of the posterior forebrain. *Development* **130**, 5579–5587 (2003).
69. Garcia-Lopez, R., Vieira, C., Echevarria, D. & Martinez, S. Fate map of the diencephalon and the zona limitans at the 10-somites stage in chick embryos. *Dev. Biol.* **268**, 514–530 (2004).

70. Houart, C., Westerfield, M. & Wilson, S. W. A small population of anterior cells patterns the forebrain during zebrafish gastrulation. *Nature* **391**, 788–792 (1998).
71. Houart, C. *et al.* Establishment of the telencephalon during gastrulation by local antagonism of Wnt signaling. *Neuron* **35**, 255–265 (2002).
72. Shimamura, K. & Rubenstein, J. L. Inductive interactions direct early regionalization of the mouse forebrain. *Development* **124**, 2709–2718 (1997).
73. Walshe, J. & Mason, I. Unique and combinatorial functions of Fgf3 and Fgf8 during zebrafish forebrain development. *Development* **130**, 4337–4349 (2003).
74. Placzek, M. & Briscoe, J. The floor plate: multiple cells, multiple signals. *Nature Rev. Neurosci.* **6**, 230–240 (2005).
75. Lee, K. J. & Jessell, T. M. The specification of dorsal cell fates in the vertebrate central nervous system. *Annu. Rev. Neurosci.* **22**, 261–294 (1999).
76. Kim, A. S., Anderson, S. A., Rubenstein, J. L., Lowenstein, D. H. & Pleasure, S. J. Pax-6 regulates expression of *SFRP-2* and *Wnt-7b* in the developing CNS. *J. Neurosci.* **21**, RC132 (2001).
77. Assimacopoulos, S., Grove, E. A. & Ragsdale, C. W. Identification of a Pax6-dependent epidermal growth factor family signaling source at the lateral edge of the embryonic cerebral cortex. *J. Neurosci.* **23**, 6399–6403 (2003).
78. Grove, E. A., Tole, S., Limon, J., Yip, L. & Ragsdale, C. W. The hem of the embryonic cerebral cortex is defined by the expression of multiple *Wnt* genes and is compromised in Gli3-deficient mice. *Development* **125**, 2315–2325 (1998).
79. Niehrs, C. Regionally specific induction by the Spemann–Mangold organizer. *Nature Rev. Genet.* **5**, 425–434 (2004).
80. Wilson, S. W. & Houart, C. Early steps in the development of the forebrain. *Dev. Cell* **6**, 167–181 (2004).
81. Kiecker, C. & Niehrs, C. A morphogen gradient of Wnt/β-catenin signalling regulates anteroposterior neural patterning in *Xenopus*. *Development* **128**, 4189–4201 (2001).
82. Nordström, U., Jessell, T. M. & Edlund, T. Progressive induction of caudal neural character by graded Wnt signaling. *Nature Neurosci.* **5**, 525–532 (2002).
83. Rhinn, M., Lun, K., Luz, M., Werner, M. & Brand, M. Positioning of the midbrain–hindbrain boundary organizer through global posteriorization of the neuroectoderm mediated by Wnt8 signaling. *Development* **132**, 1261–1272 (2005).
84. Lagutin, O. V. *et al.* Six3 repression of Wnt signaling in the anterior neuroectoderm is essential for vertebrate forebrain development. *Genes Dev.* **17**, 368–379 (2003).
85. Zhou, C. J., Pinson, K. I. & Pleasure, S. J. Severe defects in dorsal thalamic development in low-density lipoprotein receptor-related protein-6 mutants. *J. Neurosci.* **24**, 7632–7639 (2004).
86. Zeltser, L. M. Shh-dependent formation of the ZLI is opposed by signals from the dorsal diencephalon. *Development* **132**, 2023–2033 (2005).
87. McKay, I. J. *et al.* The *kreisler* mouse: a hindbrain segmentation mutant that lacks two rhombomeres. *Development* **120**, 2199–2211 (1994).
88. Schneider-Maunoury, S., Seitanidou, T., Charnay, P. & Lumsden, A. Segmental and neuronal architecture of the hindbrain of *Krox-20* mouse mutants. *Development* **124**, 1215–1226 (1997).
89. Manzanares, M. *et al.* The role of *kreisler* in segmentation during hindbrain development. *Dev. Biol.* **211**, 220–237 (1999).
90. Waskiewicz, A. J., Rikhof, H. A. & Moens, C. B. Eliminating zebrafish pbx proteins reveals a hindbrain ground state. *Dev. Cell* **3**, 723–733 (2002).
91. Dupé, V. & Lumsden, A. Hindbrain patterning involves graded responses to retinoic acid signalling. *Development* **128**, 2199–2208 (2001).
92. Maden, M. Retinoid signalling in the development of the central nervous system. *Nature Rev. Neurosci.* **3**, 843–853 (2002).
93. Trokovic, R. *et al.* Fgfr1-dependent boundary cells between developing mid- and hindbrain. *Dev. Biol.* **278**, 428–439 (2005).
94. Blair, S. S. & Ralston, A. Smoothened-mediated Hedgehog signalling is required for the maintenance of the anterior-posterior lineage restriction in the developing wing of *Drosophila*. *Development* **124**, 4053–4063 (1997).
95. Rodriguez, I. & Basler, K. Control of compartmental affinity boundaries by *hedgehog*. *Nature* **389**, 614–618 (1997).
96. Lawrence, P. A., Casal, J. & Struhl, G. The hedgehog morphogen and gradients of cell affinity in the abdomen of *Drosophila*. *Development* **126**, 2441–2449 (1999).
97. Araki, I. & Nakamura, H. *Engrailed* defines the position of dorsal di-mesencephalic boundary by repressing diencephalic fate. *Development* **126**, 5127–5135 (1999).
98. Matsunaga, E., Araki, I. & Nakamura, H. *Pax6* defines the di-mesencephalic boundary by repressing *En1* and *Pax2*. *Development* **127**, 2357–2365 (2000).
99. Scholpp, S., Lohs, C. & Brand, M. Engrailed and Fgf8 act synergistically to maintain the boundary between diencephalon and mesencephalon. *Development* **130**, 4881–4893 (2003).
100. Tepass, U., Godt, D. & Winklbauer, R. Cell sorting in animal development: signalling and adhesive mechanisms in the formation of tissue boundaries. *Curr. Opin. Genet. Dev.* **12**, 572–582 (2002).
101. Holtfreter, J. Gewebeaffinität, ein Mittel der embryonalen Formbildung. *Arch Exp Zellforsch Besonders Gewebezucht* **23**, 169–209 (1939).
The author of this classic paper suggested that differential cell affinities might be one of the driving forces in embryogenesis.
102. Duguay, D., Foty, R. A. & Steinberg, M. S. Cadherin-mediated cell adhesion and tissue segregation: qualitative and quantitative determinants. *Dev. Biol.* **253**, 309–323 (2003).
103. Nittenberg, R. *et al.* Cell movements, neuronal organisation and gene expression in hindbrains lacking morphological boundaries. *Development* **124**, 2297–2306 (1997).
104. Wizenmann, A. & Lumsden, A. Segregation of rhombomeres by differential chemoaffinity. *Mol. Cell. Neurosci.* **9**, 448–459 (1997).
105. Neyt, C., Welch, M., Langston, A., Kohtz, J. & Fishell, G. A short-range signal restricts cell movement between telencephalic proliferative zones. *J. Neurosci.* **17**, 9194–9203 (1997).
106. Redies, C. & Takeichi, M. Cadherins in the developing central nervous system: an adhesive code for segmental and functional subdivisions. *Dev. Biol.* **180**, 413–423 (1996).
107. Redies, C. *et al.* Morphologic fate of diencephalic prosomeres and their subdivisions revealed by mapping cadherin expression. *J. Comp. Neurol.* **421**, 481–514 (2000).
108. Inoue, T. *et al.* Role of cadherins in maintaining the compartment boundary between the cortex and striatum during development. *Development* **128**, 561–569 (2001).
109. Steinberg, M. S. & Takeichi, M. Experimental specification of cell sorting, tissue spreading, and specific spatial patterning by quantitative differences in cadherin expression. *Proc. Natl Acad. Sci. USA* **91**, 206–209 (1994).
Shows that mere differences in expression levels of cadherins are sufficient to render cell populations immiscible.
110. Palmer, A. & Klein, R. Multiple roles of ephrins in morphogenesis, neuronal networking, and brain function. *Genes Dev.* **17**, 1429–1450 (2003).
111. Poliakov, A., Cotrina, M. & Wilkinson, D. G. Diverse roles of Eph receptors and ephrins in the regulation of cell migration and tissue assembly. *Dev. Cell* **7**, 465–480 (2004).
112. Xu, Q., Mellitzer, G., Robinson, V. & Wilkinson, D. G. *in vivo* cell sorting in complementary segmental domains mediated by Eph receptors and ephrins. *Nature* **399**, 267–271 (1999).
113. Mellitzer, G., Xu, Q. & Wilkinson, D. G. Eph receptors and ephrins restrict cell intermingling and communication. *Nature* **400**, 77–81 (1999).
These two studies provide evidence that bidirectional Eph–ephrin signalling at rhombomere boundaries prevents cell intermingling between even-numbered and odd-numbered rhombomeres.
114. Cooke, J. E., Kemp, H. A. & Moens, C. B. EphA4 is required for cell adhesion and rhombomere-boundary formation in the zebrafish. *Curr. Biol.* **15**, 536–542 (2005).
115. Rhinn, M., Dierich, A., Le Meur, M. & Ang, S.-L. Cell autonomous and non-cell autonomous functions of Otx2 in patterning the rostral brain. *Development* **126**, 4295–4304 (1999).
116. Kopan, R. & Turner, D. L. The Notch pathway: democracy and aristocracy in the selection of cell fate. *Curr. Opin. Neurobiol.* **6**, 594–601 (1996).
117. Mathis, L., Sieur, J., Voiculescu, O., Charnay, P. & Nicolas, J. F. Successive patterns of clonal cell dispersion in relation to neuromeric subdivision in the mouse neuroepithelium. *Development* **126**, 4095–4106 (1999).
118. Glickman, N. S., Kimmel, C. B., Jones, M. A. & Adams, R. J. Shaping the zebrafish notochord. *Development* **130**, 873–887 (2003).
119. Guthrie, S., Prince, V. & Lumsden, A. Selective dispersal of avian rhombomere cells in orthotopic and heterotopic grafts. *Development* **118**, 527–538 (1993).
120. Pourquié, O. Vertebrate somitogenesis: a novel paradigm for animal segmentation? *Int. J. Dev. Biol.* **47**, 597–603 (2003).
121. Aulehla, A. & Herrmann, B. G. Segmentation in vertebrates: clock and gradient finally joined. *Genes Dev.* **18**, 2060–2067 (2004).
122. Lynch, J. & Desplan, C. 'De-evolution' of *Drosophila* toward a more generic mode of axis patterning. *Int. J. Dev. Biol.* **47**, 497–503 (2003).

Acknowledgements

We thank D. Wilkinson for helpful comments on the manuscript, and both the Medical Research Council and the Wellcome Trust for supporting our work. We apologize to all researchers whose work we could not cite owing to space limitations.

Competing interests statement

The authors declare no competing financial interests.

Online links

DATABASES

The following terms in this article are linked online to:

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene

Dlx | *Emx* | EphA4 | *fgf8* | *Gbx2* | *Hoxa2* | *Hoxb1* | *hoxb1a* | *Irx2* | *Krox20* | *Lfng* | Nodal | *Otx2* | *Pax6* | *pou2* | *rfng* | SFRP2 | SHH | *Six3* | *Wnt1* | *Wnt8b*

FURTHER INFORMATION

MRC Centre for Developmental Neurobiology: http://www.kcl.ac.uk/depsta/biomedical/mrc/index.php?page=http://www.kcl.ac.uk/depsta/biomedical/mrc/

Access to this interactive links box is free online.