Recent advances in neural development

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Abstract

A surprisingly small number of signalling pathways are used reiteratively during neural development, eliciting very different responses depending on the cellular context. Thus, the way a neural cell responds to a given signal is as important as the signal itself and this responsiveness, also called competence, changes with time. Here we describe recent advances in elucidating the signalling pathways that operate in brain development.

Introduction and context

One of the most formidable challenges in biology is to understand the generative program underlying the development of a functional nervous system and, in the case of vertebrates, the astonishingly complex structure of the brain. The neuroepithelium that will make the brain and spinal cord is induced early in development, partly through inhibition of anti-neuralizing signals. In vertebrates, the emerging epithelial sheet - the neural plate - is then patterned coarsely along its anteroposterior (AP, head-to-tail) and dorsoventral (DV, back-to-belly) axes by gradients of secreted factors (morphogens) that specify different regional neural fates in a dose-dependent fashion. Subsequently, regional identities become stabilized through transcriptional feedback and through the establishment of cell-tight compartments. The neural plate rolls up and compacts to form a neural tube that displays increasingly pronounced bulges, constrictions and flexures - the first indication of the morphological complexity of the central nervous system (CNS) at later stages (Figure 1).

Local signalling centres are established within the neuroepithelium, often along the boundaries between compartments, which refine the pattern of neural subdivisions by releasing diffusible signalling factors. A surprisingly small set of signalling factors is employed reiteratively throughout development, and different populations of cells may respond to the same signal very differently, a phenomenon called 'differential cellular competence'. Eventually, neural identities become determined when neural progenitors exit the cell cycle and differentiate into mature neurons that form dendrites and project axons to establish the complex connectional architecture of the CNS. Understanding the developmental history of cells in specific regions of the emerging brain will provide us with more rational and targeted strategies to produce these cells in a Petri dish from embryonic stem cells.

The initial step in CNS development in vertebrates - the induction of a neural plate from the embryonic ectoderm - occurs early in embryogenesis before the onset of gastrulation. In the 1990s the 'default model' for neural induction was proposed: all ectodermal cells will become neural unless they are exposed to epidermis-inducing bone morphogenetic proteins (BMPs) [1–3]. Thus, neural fates are induced either by the mere absence of BMP signals (by default) or by an active inhibition of the BMP signalling pathway. Over the past 15 years, it has been shown that embryos throughout the animal kingdom produce inhibitory factors that sequester BMPs in the extracellular space and relieve cells from their anti-neuralizing effect, thereby inducing neural identity [4,5].

During gastrulation, a crude pattern is established within the neural plate by gradients of signalling factors that determine AP polarity (fibroblast growth factors (FGFs),

Figure 1. Lateral view of embryonic vertebrate (chick) brain
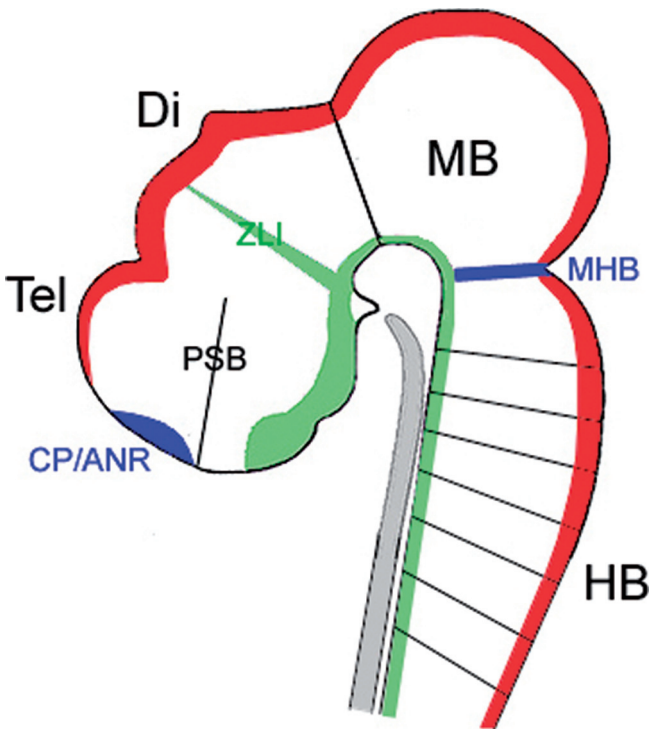
Principal signalling centres are highlighted in green (floor plate, basal forebrain, zona limitans intrathalamica (ZLI) - Shh expression), red (roof plate - BMP and Wnt expression) and blue (midbrain-hindbrain boundary (MHB), anterior neural ridge/commissural plate (ANR/CP) - FGF expression). Note that the pallial-subpallial boundary (PSB) and the boundaries between rhombomeres in the hindbrain (HB) have also been suggested to exert signalling functions. The notochord (light grey) is a non-neural signalling centre that regulates ventral neural patterning. Di, diencephalon; MB, midbrain; Tel, telencephalon.

retinoic acid, secreted signalling proteins of the Wnt family) and mediolateral polarity (BMPs, members of the Hedgehog family) by inducing the expression of region-specific transcription factors in a dose-dependent fashion [6–13]. In many cases, the borders between domains of transcription factor expression are then sharpened by the mutual repression of pairs of factors. For example, the expression domains of the homeobox genes *Otx2* in the prospective midbrain and *Gbx2* in the anterior hindbrain region initially overlap [14], but mutual repression between the two transcription factors encoded by these genes results in a binary choice, with cells exclusively expressing either *Otx2* or *Gbx2* [15–18]. Furthermore, cells in adjacent regions may start to express different sets of surface molecules, resulting in an enhanced affinity between cells within a region, decreased affinity and miscibility with cells from neighbouring regions, and the formation of a sharp

regional interface - similar to the formation of a phase interface between oil and water [19].

Occasionally, a regional interface becomes a cell-tight boundary that confines cells to lineage-restricted compartments; this is best exemplified in the hindbrain, which consists of a series of compartments called rhombomeres [19,20]. Apart from stabilizing emerging regionalization, boundaries often appear to function as local organizers, specialized cell populations that influence the development of their flanking regions by secreting molecular signals [11,19,21]. For example, the boundary between midbrain and hindbrain (MHB) induces the tectum anteriorly and the cerebellum posteriorly by releasing FGF8 [9,11,16,18]. Thus, the themes of (1) patterning by diffusible signalling factors, (2) mutual repression of transcription factors and (3) boundary/compartment formation are reiterated at multiple stages of brain development, resulting in a progressively refined subdivision of the neuroepithelium.

While the assignment of regional identities is under way, the neuroepithelium undergoes a no less complex series of morphological transformations. The neural plate rolls up and its borders fuse to form a neural tube that displays increasingly pronounced constrictions and bulges (some of which correspond to the boundaries and compartments discussed above) [19,22,23]. At present, little is known about the molecular dynamics underlying brain morphogenesis, but differential growth is likely to be one of the driving forces, and some of the signalling factors secreted by local organizers act as growth factors in addition to their role in patterning. Whereas AP patterning of the neural tube continues to be under the influence of several discrete local organizers (such as the MHB), DV patterning is regulated by two continuous signalling centres that stretch along almost the entire neural tube: the floor plate at the ventral midline that controls ventral identity by secreting Sonic hedgehog (Shh) [24–26] and the roof plate at the dorsal midline that emits BMPs and Wnts [6,27,28].

Neural progenitors are kept in a proliferative state until, at the onset of neurogenesis, their cell cycle lengthens and both postmitotic neurons and radial glial cells are produced. Basic helix-loop-helix transcription factors such as NeuroD and the neurogenins, together with the Notch signalling pathway, are key regulators of this process [29,30]. Throughout the brain, neurons become organized to form either nuclei (in the diencephalon, tegmentum and brain stem) or layers (cortex, tectum and cerebellum). In the cerebral cortex, layering is achieved by the sequential radial (outward) migration of newborn neurons that are generated in the ventricular zone.

Once the basic architecture of the brain has been established, some postmitotic neurons become redistributed by tangential migratory processes. For example, GABAergic neurons that originate in the basal forebrain migrate dorsally into the cortex in mammals [31]. Finally, neurons form dendrites and project axons to targets within the brain and in the periphery. Axons are guided by extracellular cues that can act as attractants (netrin and its receptor DCC) or as repellants (ephrins and Eph receptors, netrin and its receptor UNC5, semaphorins and plexin receptors, Slit and its receptor Robo) [32–35]. Sensory input is often represented in an orderly fashion in various brain structures. The best example of such topographic mapping is the projection pattern of retinal axons into the optic tectum, where the two axes of the retina (nasal-temporal, ventral-dorsal) correspond to projection targets along the caudal-rostral and medial-lateral axes of the tectum, respectively (Figure 2). This geometric organization is achieved, at least in part, by the graded expression of ephrins within the tectum and of Eph receptors on retinal axons [36,37]. It is likely that these gradients of expression are set up by the gradients of signalling factors that pattern the neuroepithelium at earlier stages, highlighting a direct link between early patterning and functional brain architecture.

Major recent advances
Neural induction
Support for the default model of neural induction has recently come from experiments in frog embryos in which the simultaneous depletion of three BMP inhibitors resulted in the absence of a neural plate [38] while the simultaneous depletion of three BMPs resulted in massive neural induction [39]. At the same time, however, experiments in chick suggested that prospective neural cells require activation by FGFs before BMP inhibition [9,40,41]. This is consistent with the results of other groups who have demonstrated that BMP inhibition is required, but not sufficient, for neural plate formation in frog and fish embryos [42–45].

Crucial steps in embryogenesis, such as neural induction, are often governed by multiple parallel pathways, providing a safety mechanism that ensures increased fidelity in cellular decision-making. In addition to BMP inhibition and FGF signalling, both activation and inhibition of the Wnt pathway have also been implicated in neural induction [46–49]. The De Robertis lab and others have shown that BMP, FGF/MAP kinase and Wnt pathways are all integrated at the level of Smad1 phosphorylation (Figure 3), providing an elegant explanation for the neural-inducing and neural-inhibiting activities of these pathways [46,50,51]. The integration

Figure 2. Topographic mapping in the retinotectal pathway
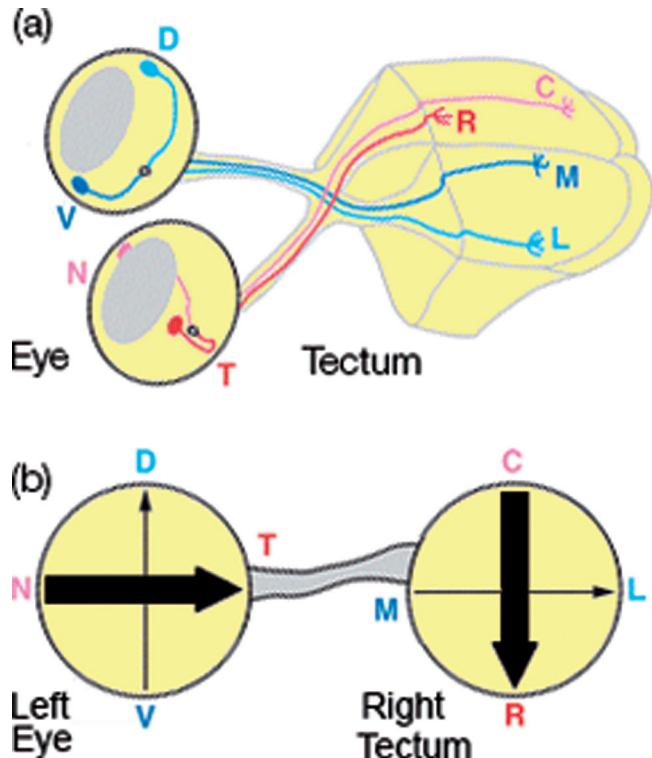
(a) Axons originating from dorsal (D) aspects of the retina project into the lateral (L) tectum (bright blue) whereas axons from the ventral (V) retina project into the medial (M) tectum (dark blue). Nasal (N) retinal axons target caudal (C) areas of the tectum (pink) whereas temporal (T) ones target rostrally (R, red). (b) Simplified, vectorial depiction of retinotectal projection patterns.

of three different pathways safeguards the formation of neural cells in a spatially and temporally restricted manner in the embryo. However, even the simultaneous manipulation of BMP, FGF and Wnt signalling may not be sufficient to induce neural tissue in all experimental systems, suggesting the presence of other, as yet unidentified, neural-inducing signals [45].

In both chick and frog, the FGF target gene *churchill* (*chch*) was proposed to block BMP signalling by encoding a zinc finger transcription factor that induced the expression of the Smad inhibitor Sip1 [52]. However, a recent structural analysis of Chch has indicated that the protein is unlikely to bind to DNA directly, raising the question of how Chch induces Sip1 - possibly by interacting with another DNA-binding cofactor [53].

Patterning
In the 1930s, Otto Mangold proposed that the early neural plate is already subdivided into multiple AP

Figure 3. Integration of BMP, FGF and Wnt signalling at the level of Smad1 during neural induction
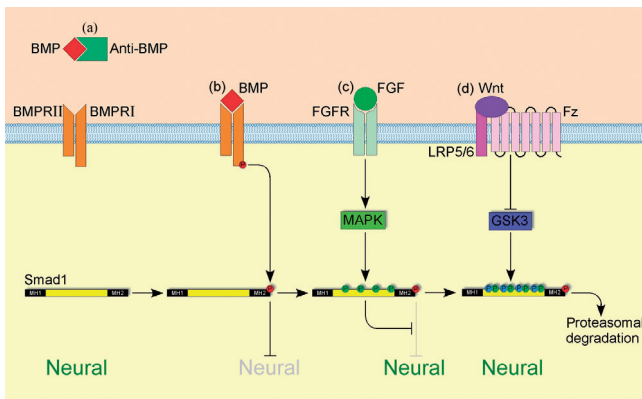
(a) Anti-BMPs (such as Chordin and Noggin) prevent BMPs from binding to their receptor complex; Smad1 remains inactive; neural identity is induced. **(b)** Binding of a ligand to a BMP receptor complex results in transphosphorylation of the BMP type I receptor by the BMP type II receptor and the subsequent phosphorylation of Smad1 in its carboxy-terminal MH2 domain; this activated Smad1 forms a complex with co-Smads, translocates to the nucleus and blocks neural identity. **(c)** Activation of the mitogen-activated protein kinase (MAPK) pathway by FGF (or by hepatocyte growth factor (HGF) or insulin-like growth factor (IGF)) signalling results in phosphorylation of Smad1 in its linker region (yellow); this phosphorylation blocks the anti-neuralizing effect of activated Smad1. **(d)** Phosphorylation in its linker region primes Smad1 for phosphorylation by glycogen synthase kinase 3 (GSK3); doubly phosphorylated Smad1 is then targeted for degradation in the proteasome.

domains [54]. This model was called into question by many studies that found a high level of regional plasticity within the neural plate, indicating that it is a relatively naive sheet of cells. A recent fate-mapping study in zebrafish has, however, revealed a high level of determination within subregions of the neural plate during gastrulation (in particular in the area of the presumptive prethalamus) [55]. Analysis of the promoter of a *Xenopus* orthologue of the Drosophila gene*caudal*, which encodes a homeodomain transcription factor expressed in the posterior neural plate, has revealed the presence of multiple regulatory elements that are able to integrate BMP, FGF and Wnt signals, providing evidence for the idea that several pathways interact not only during neural induction but also during the establishment of the posterior CNS [56]. Anteriorly, the homeobox transcription factor Six3 protects forebrain identity by repressing the posteriorizing activity of Wnt1 [57,58].

Local signalling centres are likely to release a secreted signal in a more or less symmetrical fashion. Yet, the response to the same signal on either side of the

signalling centre is often strikingly asymmetrical. For example, why do cells on either side of the MHB interpret the same signal, FGF8, differently by forming a tectum anteriorly and a cerebellum posteriorly? A recent study has highlighted a central role for the homeobox transcription factor Irx2 in conferring competence upon cells posterior to the MHB to form cerebellum in response to FGF8 [59]. We and others were able to show that the zona limitans intrathalamica (ZLI) in the posterior forebrain is also a local signalling centre that regulates thalamic development by emitting Shh. Irx3, a close relative of the competence factor Irx2, is expressed posterior to the ZLI and determines the 'thalamic response' of the posterior cells to Shh [60]. The expression domains of these competence factors are established early in development, at neural plate stages, linking the coarse pre-pattern that is set up during gastrulation with the later refinement of this pattern. The boundaries between hindbrain rhombomeres have also been shown to exert signalling activity by producing Wnts that regulate the pattern of neurogenesis within the rhombomeres [61,62].

Two studies from the Partanen lab have shown that FGF receptor signalling is essential not only for patterning in the MHB area, but also for maintaining the integrity of the MHB itself [63] and for promoting progenitor proliferation [64]. These studies emphasize the multi-functionality of secreted signalling factors.

Morphogenesis
Wnt proteins are able to activate two alternative intracellular pathways. One is the 'canonical' Wnt pathway that results in stabilization of β-catenin and its translocation to the nucleus, where it associates with various cofactors to activate the transcription of target genes [65]. The other is a 'noncanonical' Wnt pathway that interacts with the cytoskeleton independently of transcription and regulates epithelial cell polarity [66,67]. It has become increasingly clear that the noncanonical branch of the Wnt pathway (activated by Frizzled3 and Frizzled6) is required for the morphogenetic process of neural tube closure [68,69].

Neuronal phenotype
Postmitotic neurons acquire characteristic neurotransmitter phenotypes depending on their gene-expression profile. For example, Otx2, which acts as a pre-patterning factor at earlier stages, promotes glutamatergic differentiation and represses GABAergic differentiation in the thalamus [70]. Similarly, somatosensory neurons in the hindbrain are replaced by viscerosensory relay neurons in mice lacking the transcription factor Lbx1 [71]. A recent paper from the Briscoe lab [72] has shown that the

mutual repression of transcription factors is not only important for the establishment of neuroepithelial subregions in the hindbrain, but also determines the decision between a visceral motor neuron and a serotonergic neuron fate. Thus, early brain patterning and later neuronal function are inherently linked by the characteristic gene-expression profile of a given brain area.

Axon guidance and map formation

A host of novel molecular players and interactions in axon guidance have been identified [73,74], with mutagenesis screens in zebrafish proving a particularly useful tool for such gene-mining projects [75]. As with patterning and the establishment of neuronal phenotypes, the expression or absence of single transcription factors can have a profound influence on axon projections and the formation of functional synapses [76]. Recently, the formation of the facial somatosensory map was shown to depend on the *Hox*-gene-regulated rhombomeric organization of the hindbrain, providing yet another example of a direct link between early brain regionalization and the establishment of functional architecture [77].

Hodge *et al.* [78] have shown that the regionalized expression of BMP4 in facial structures of the mouse embryo regulates gene expression in trigeminal sensory neurons and, as a consequence, influences the spatial projection pattern of these neurons. This study indicates that target-derived signals play a crucial role in shaping neuronal architecture.

Future directions

Neural induction is still a hotly debated topic, but it seems likely that FGFs and other signals mediate the earliest steps of this process and that the inhibition of BMPs (probably in combination with Wnt inhibitors and ongoing FGF signalling) serves to stabilize the early-induced neural fate [79]. It has become increasingly clear that secreted signalling factors perform different functions at different developmental stages, not only regulating patterning [56,60,63,64,80], but also proliferation [64], the acquisition of neuronal phenotypes [64,78] and even axon guidance [6,8,9,81–83].

Our deepening knowledge of the molecular processes that establish specific subregions of the brain allows us to mimic these steps in a Petri dish in order to drive embryonic stem cells along certain predictable developmental routes. So far, motor neurons [84], telencephalic precursors [85], dopaminergic midbrain neurons [86] and cerebellar granule cells [87] have been generated *in vitro* and in some cases have been shown to integrate

successfully into the corresponding structures of a developing brain. Thus, basic research in developmental neurobiology has opened up new avenues and can offer more specific, target-oriented approaches in producing stem cells for therapeutic purposes.

Abbreviations

AP, anteroposterior; BMPs, bone morphogenetic proteins; *chch*, the FGF target gene *churchill*; CNS, central nervous system; DV, dorsoventral; FGFs, fibroblast growth factors; MHB, boundary between midbrain and hindbrain; Shh, Sonic hedgehog.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

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