Chapter 1

Sonic Hedgehog Signalling in Dorsal Midline and Neural Development

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Abstract

Sonic hedgehog is a secreted morphogen involved in patterning of a variety of structures and organs in vertebrates. In this chapter we focus on its role in the development of the floor plate and in the events that pattern and configure the shape and size of the central nervous system.

The Hedgehog Pathway

Hedgehog (Hh) proteins comprise a family of secreted morphogens that exert short and long range actions essential for patterning a variety of structures during animal embryogenesis.[1-5] On the course of their maturation process, Hh proteins undergo an autocatalytic cleavage that renders the active N-terminal polypeptide, which gains hydrophobicity by cholesterol and palmitate additions important for modulating the range of action. Short-range signalling involves tethering by cholesterol and up-regulation by Hh of its own receptor, Patched (Ptc), which is supposed to limit the range of action by ligand sequestration. Signalling at a distance depends on Dispatched, a transmembrane sterol-sensing protein necessary for release of Hh from the sending cell, and requires heparan sulfate proteoglycans and enzymes for heparan sulfate biosynthesis. After sensing the morphogen concentration, perhaps by perceiving the ratio of liganded to unliganded Ptc, the field of receiving cells modulate the activity of different forms of the latent cytoplasmic zinc-finger transcription factors Ci (Drosophila) and Gli (vertebrates), which ultimately turn-on different sets of target genes according to the distance to the morphogen source. In fact, Ci can display activating and repressing forms: the full-length transcriptional activator and the repressing N-terminal fragment generated by proteolysis (CiR). In vertebrates, the three homologues of Ci have activating properties, and only the proteolytic N-fragments of Gli2 and Gli3 appear to function as potent transcriptional repressors.

The mechanism by which Hh signalling is transduced is complex and subtly modulated, and actually involves a release of repression. In the absence of Hh signalling, the kinesin-like protein Costal-2 (Cos2) is stably associated with Ci. In this complex, Cos2 may mediate the scaffolding of a series of kinases that sequentially phosporylate Ci. Phosphorylated Ci then undergoes proteolysis, rendering the repressor form CiR. At the same time, Su(fu) (Suppresor of fused) inhibits the transcriptional activity of full-length Ci.

When Hh binds Ptc, the transmembrane protein Smoothened (Smo) is released from a state of repression. If fully active, Smo recruits Cos2 and Fused (Fu). In this state, Fu is stabilized and inhibits Su(fu), leading to positive transcriptional activity of full-length Ci. Mean-

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while, recruitment of Cos2 by active Smo results in blockage of Ci phosphorylation and ceasing of CiR production. Therefore, both actions result in the full range response of the transcriptional targets of Hh signalling. But if Smo is partially active, Fu is not stabilized, the activity of full-length Ci remains suppressed and only CiR formation is stopped, resulting in a partial response of a subset of target genes (for revision of Hh transport, release, reception and transduction, see refs. 6-10). Three distinct members of the Hh family have been characterized in vertebrates: *sonic (shh), indian (ihh)* and *desert (dhh).* We will focus on the role of *shh* in floor plate and neural development.

Shh Signalling and Floor Plate Formation

The floor plate (FP) is a modified glial structure located in the ventral midline of the vertebrate neural tube. It constitutes an important source of signals involved in dorsoventral (D/V) neural patterning, proliferation and survival of neural precursors, and attraction and repulsion of axons in route to their destination.[11-16] It is generally accepted that as an anatomical structure, it extends from the ventral midbrain to the tail region. However, *shh* and *netrin-1*, typical FP markers, are also detectable in the diencephalon of all vertebrates, suggesting that the ventral midline of the anterior brain share FP properties.

Although vertebrates display some variation in the molecular dynamics, a consensus picture shows the FP as composed of three longitudinal regions, one medial (MFP) flanked by two lateral ones (LFP). In mouse and zebrafish, *shh* is only expressed by the MFP, while the winged-helix transcription factor *foxa2* (formerly known as *hnf3β*) is present in both populations. In chicken, MFP and LFP cells initially express both markers, but *foxa2* later becomes restricted to MFP, while some *shh* expression remains in the LFP.[17-18] However, an issue that cannot be circumvented is that apart from the variable expression of *shh* or *foxa2*, the LFP expresses neural markers, like the transcription factors *sox1* and *nkx2.2. Sox1* is a general neuroepithelial marker necessary and sufficient to maintain panneural properties of neural progenitor cells.[19] *Nkx2.2* is expressed by the progenitors of V3 interneurons and oligodendrocytes and it is necessary for their differentiation in the ventral neural tube.[20-22] Besides, the LFP is constituted by seudostratified neuroepithelium, different from the polarized cell structure characteristic of the MFP.[23] Therefore, the LFP could be seen as part of the ventral neural tube domain where neural progenitors are deciding their fate. Indeed, the feature that has been used to define the FP is the expression of *shh* or *foxa2*, which might be insufficient to determine a real functional unit. In conclusion, the division between LFP and MFP could be seen as a simplistic interpretation from the expression patterns of some markers.

The origin of the FP has been the subject of great controversy. The canonical model poses that it is induced on the neural ectoderm by vertical signals from the underlying notochord. This was founded on the observations that the avian FP cannot develop after removal of the notochord but appears ectopically after grafting notochordal tissue onto the lateral or dorsal regions of the neural tube.[24] Evidence from different vertebrate species highlighted Shh as the signal responsible for inducing FP in a typical short-range action that requires direct contact with the notochord and exposition to high concentrations of the morphogen. Lower levels of Shh secreted from the FP would then induce diverse cell types in the neighbouring ventral spinal cord, including motor neurons and interneurons, in a dose-dependent way.[3-4,25-29]

In mouse, Shh signalling appears to be essential for FP development, since targeted disruption of *shh* blocks FP differentiation without impairing the early development of the notochord.[30] However, genetic manipulations in zebrafish undermined the protagonist role of Hh on FP development, because mutations of members of this pathway only impaired the development of the LFP. In this species, Nodal signalling was proposed to induce MFP and then, secreted Hh from the MFP would induce LFP. Nevertheless, in the absence of Nodal signals, some cells can acquire a mixed MFP-LFP character at later stages. It remains to be elucidated whether Hh could play a role in this late differentiation process. Strikingly, *smu (smo)* mutants, which have a general blockade of Hh signalling, ultimately show gaps in the MFP, suggesting

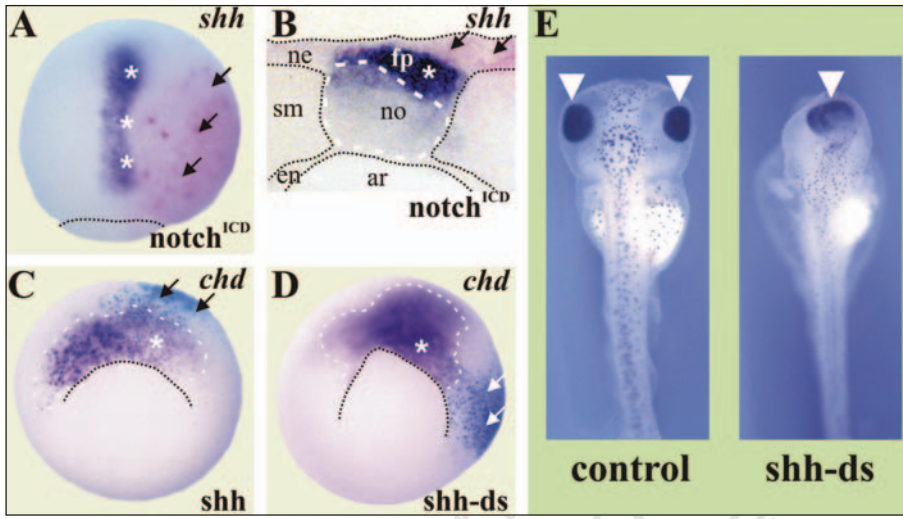

Figure 1. Notch and Shh signalling in Xenopus dorsal midline development. A,B) Notch increases *shh* expression in the FP, which is expanded at the expense of the notochord. A, dorsal view of an early neurula injected with mRNA encoding the intracellular domain of Notch (notch^{ICD}), a constitutively active form independent of ligand binding. Anterior is oriented to the top. Cells that inherited the injected mRNA were revealed by magenta immunostaining of the myc-tag epitope fused to Notch^{ICD}. The level of *shh* transcripts (purple staining) is increased on the injected side (right, green asterisks). The dotted line demarcates the nearly closed blastopore. B) transverse section of the same embryo shown in (A). The shh expression domain in the FP (purple staining) is expanded (green asterisk), while the notochord is reduced on the injected side (right). The dotted red line demarcates the contour of the notochord (no). ne, neuroectoderm; sm, somitic mesoderm; en, endoderm; ar, archenteron. C,D) Shh signalling restricts the number of notochord precursors. C) vegetal view of an early gastrula injected with *shh* mRNA. Notochordal precursors are decreased in the organiser on the injected side (right, red asterisk), as evidenced by the expression of the notochordal marker *chordin* (*chd,* purple staining). D) vegetal view of a mid gastrula where *shh* function was knocked-down by injection of *shh* double-stranded RNA (shh-ds). *Chd* expression (purple staining) shows that the number of notochordal precursors is increased in the organiser on the injected side (right, green asterisk). In (C) and (D), dorsal is oriented to the top, turquoise staining reveals the coinjected lacZ lineage tracer, and the dotted line demarcates the dorsal blastopore lip. E) Dorsal views of a control tadpole (left) and a sibling embryo showing cyclopia as the result of knocking-down *shh* function with shh-ds RNA (right). Eyes are pointed by arrowheads. Embryos shown in (E) were extracted from López et al.[38]

that Hh, although not necessary for MFP specification in zebrafish, is later required for maintaining the phenotype of these cells or for their survival.[31-32,18]

In the last years, a second model of FP development challenged the idea of its neural origin. New experiments on birds led to the proposal that Hensen´s node (equivalent to the amphibian's Spemann´s organiser) generates both midline structures. The reason why the FP does not develop after the removal of the notochord is that the FP precursors are removed together.[23] Thus, the FP would be a mesodermal derivative rather than a neuroectodermal one. Indeed, the groundwork for this idea can be traced back to the pioneering experiments of Spemann and Mangold,[33] who clearly demonstrated that the implanted amphibian dorsal lip differentiates into notochord and FP in the trunk. Fate maps of the embryonic shield, the teleost equivalent of the Spemann's organiser, also established that this region contributes to both structures.[34-35]

The Hensen´s node can be subdivided into three morphological and functional domains.[36] The caudal-most tip (named zone c by the authors) ends at the axial-paraxial hinge and contains *foxa2+ shh-* cells closely packed and randomly arranged. The medial part (zone b) lies on the median pit and contains *foxa2+ shh+* cells. An outline of two cell-layers becomes apparent

in this zone: an epithelial-like layer, presumably containing the FP precursors, which already shows a columnar arrangement, and the deep layer, delineating the future notochord, with cells distributed at random; yet there is no clear separation between both structures. The rostral portion of the node (zone a) contains *foxa2*+ cells and more *shh*+ cells than zone b. Here, distinction of the notochord and FP is more clearly defined, but they are still in close association, although already separated by a basement membrane.

If zone b is removed, caudalward movement of zone c still occurs. However, the embryos then bear an interruption of midline cells (notochord and FP) at the trunk level. The stretch of neural tube formed consequently is smaller and is devoid of FP and motorneurons. Noteworthy, at more caudal levels, the midline cells and the neural tube resume normally. But if zone c is removed, caudalward movement of the node ceases. The neural tube formed posterior to the excision lacks notochord and FP, and it is completely dorsalised. On the basis of these observations, the authors suggest that zone c contains self-renewing cells with the potential to develop either as notochord or FP. However, grafting experiments demonstrated that although zone b contributes to all midline cells caudal to the level of the graft, zone c normally provides very few cells to the caudal FP.[26,36] Thus, although zone c, as a source of stem cells, can compensate for notochord and FP precursors when zone b is ablated, it is more likely that the bipotential, self-renewing precursors of FP and notochord are mainly found in zone b during normal development. This does not rule out that commitment to either fate also begins in this zone. Several groups have shown that Shh is a potent mitogen (see below). It would be interesting to test if Shh, whose expression is evident in zone b, is promoting mitosis of midline precursor cells. All together, the node could be considered as a functional cell niche.

The hypothesis that the notochord and the FP arise from a population of pluripotent precursors located in the vertebrate´s organiser has been strongly supported by genetic evidence. In zebrafish and *Xenopus*, Delta-Notch signalling executes a binary cell-fate decision, promoting FP specification at the expense of the notochord.[37-38] In zebrafish *ntl* mutant embryos the notochord does not develop and the MFP is widened.[39]

In *Xenopus*, Notch signalling enhances *shh* expression in FP precursors (Fig. 1A,B), and secreted Shh represses notochord specification (Fig. 1C,D).[38] and expands the FP (Rosato-Siri et al, unpublished results). Therefore, Shh would amplify the effects of the binary decision initially promoted by active Notch, resulting in an even larger population of specified FP precursors in detriment of the notochord. This mechanism could in part underlie a previously unrecognised role of Shh as FP inducer.[38] This is consistent with the normal profile of *shh* expression during amphibian or avian development. Although *shh* transcripts are detectable both in notochord and FP precursors, expression becomes significantly higher in the latter during gastrulation.[38,40] Thus, the specification of the different midline fates may be linked to a differential regulation of the *shh* gene. If cells are committed to FP fates by active Notch, *shh* would be more actively transcribed. Interestingly, the dissection of the regulatory regions of the mouse *shh* gene has uncovered two enhancers that direct expression to FP, one upstream of the coding region (SFPE1) and the other within intron2 (SFPE2).[41] Strikingly, deletion of the proximal region of SFPE1 unmasks a potent notochord enhancer (SNE), whereas expression in the FP decreases substantially. It will be interesting to determine whether this region contains binding sites for repressors that restrict notochordal expression and if the complete SFPE1 underlies a mechanism for switching notochordal to FP expression. Besides, *foxa2* function is probably required by SNE but not by SFPE1 activity. On the other hand, SFPE2 contains a sequence of significant homology between mouse, chicken and zebrafish, which harbours binding sites for Foxa2 and homeodomain transcription factors.[42] When transgenes containing trimers of this sequence were assayed, expression of the reporter was then found in the notochord in addition to the FP. Intriguingly, while the Foxa2 binding sites are necessary for notochord and FP expression, the homeodomain binding site is required only for FP expression. All these evidences suggest that expression of *shh* in the notochord and the FP is controlled by shared and independent mechanisms.

Despite growing support for the hypothesis that the vertebrate´s organiser contributes significantly to FP formation, some disagreement still persists. In the mouse embryo, before *shh* expression begins in the central nervous system (CNS), transcripts are found in the ventral (mesodermal) layer of the node, and rostrally in the notochordal plate. Because expression was noticed neither in the dorsal layer of the node or in the ventral midline of the more rostral neural plate, it was argued that the mouse FP does not derive from the node but is induced by the canonical signal from the notochord.[42] However, the idea that the notochord and part of the FP share embryonic origin is not incompatible with different patterns of gene expression once both populations have been committed to their respective fates in the node. In fact, cell-lineage tracing has shown that descendants of the dorsal layer of the mouse node populate the FP[43-44] and loss-of-function of delta-1 in mouse results in an excess of FP cells, while the notochord is reduced.[45] Although the opposite activities of Delta signalling in dorsal midline development in mouse and anamniotes embryos are intriguing, in both cases they underscore the existence of a population of cells in the organiser with equal potential to develop either as FP or notochord.

Recent evidence from avian embryos have resolved important discrepancies between the two models of FP formation. Much of this understanding comes from considering the different cell populations that compose this structure, which allowed new questions about the role of *shh* to be addressed. Careful analysis of molecular markers in quail-chick chimeras, where the chick Hensen's node was replaced by its quail counterpart, demonstrated that the MFP derives from the node, while the LFP derives from the neural plate.[17] Utilising lineage tracing, two areas of FP precursors in the chick epiblast have been distinguished; one anterior to the Hensen's node (prenodal epiblast, originally designated as "area a" by the authors), which gives rise to the earliest-forming FP in the cephalic region, and the other one in the Hensen's node, whose descendants later populate the posterior FP and the notochord.[46] Thus, the anterior FP (AFP) would be of neural origin. Although the authors did not address the issue of whether the node-derived population becomes incorporated into the LFP or MFP, they are presumably observing the medial component, as demonstrated by Charrier et al.[17]

Notochord and MFP grafts are able to induce a complete ectopic FP in the avian neural tube but with temporal and spatial restrictions. While a supernumerary LFP appears at any stage of the window tested and throughout the full length of the caudal neural tube exposed to induction, MFP is induced only in the posterior-most region of host embryos younger than 15 ss. In addition, the MFP graft needs a very close contact with the host neural epithelium to induce MFP. In contrast, Shh can induce only LFP in the neural ectoderm. Thus, Shh alone is insufficient to transform neural cells into MFP in the avian embryo.[17] Other factors provided by the notochord, presumably BMP antagonists, may be acting in cooperation with Shh to fulfil this process.[47]

The AFP is rapidly induced on the neural ectoderm by a vertical contact with the nascent prechordal mesoderm while passing beneath the prenodal region of the epiblast. Prechordal mesoderm expresses *shh* and *nodal-1.* Shh alone is sufficient to induce FP markers in prenodal epiblast explants, but only at high concentrations; when the explants are exposed to low concentrations of Shh in the presence of Nodal 1, a robust induction of FP markers is observed. Thus, Nodal and Shh signalling may cooperate during the early and rapid induction of the AFP by the prechordal mesoderm.[46]

Interestingly, an area analogous to the prenodal epiblast may exist in *Xenopus*. It is conformed by an arc of *hairy-2a+* cells in the dorsal noninvoluting marginal zone and marks the earliest signs of FP induction.[48] It will be interesting to elucidate whether the FP phenotype of these cells is induced by Shh and Nodal signals secreted by the prechordal plate, as was proposed for the avian embryo.

At this point, a main conclusion can be raised: most of the embryonic models studied so far suggest that the specification of the FP and notochord start earlier than previously recognised, challenging the canonical model where notochord induces FP. The main disagreement resides in

the absolute requirement of *shh* for FP development in mouse, as opposed to its sole role as LFP inducer in zebrafish. An integrative model for FP development can be synthesised as follows:

1. The anterior FP, of neural origin, would be induced early on the prenodal epiblast by Shh with the cooperation of Nodal, both emanating from the prechordal mesoderm that passes beneath.
2. The MFP, located posteriorely, and primarily of mesodermal origin, would be induced within the organiser, before the segregation of notochord and MFP precursors, in a binary switch triggered by Delta-Notch that favours FP fates at the expense of the notochord. This enhances *shh* expression in MFP precursors, and secreted Shh contributes to repress the notochordal fate and amplifies the switch. In turn, specified MFP precursors populate the midline of the neural plate. It remains to be elucidated whether Delta-Notch signalling modulates *shh* expression in amniote embryos, but *shh* is essential for the induction of FP in mouse. Other questions must be answered; for example, which are the molecules that pattern the scattered expression of *delta-1* in the organiser, which initially would define the distribution of MFP and notochord precursors.[48] It will be interesting to investigate whether Nodal signalling is involved in this process or acts independent of the Notch switch, given the absolute requirement of Nodal for MFP development in zebrafish. In addition, some MFP cells may be induced on the neural ectoderm by node derived MFP in close contact with the neural plate, but Shh alone is insufficient for this process and would require BMP antagonists derived from the notochord.
3. Consequent to Notch activation, MFP secretes high levels of Shh, leading to the short-range induction of the LFP on the neighbouring neural plate.

Shh in Neural Development

Ventral Neural Patterning

Shh has been classically considered as an inducer of different types of ventral neurons in the spinal cord, the phenotype of which varies according to the morphogen concentration, depending on the distance from the source in the FP. These kinds of neurons are arrayed from ventral to dorsal as follows: V3 interneurons (the closest to the FP), motorneurons (MN), V2, V1 and V0 interneurons, the latter at the level of the intermediate neural tube.[49] During the specification of ventral neural fates in the spinal cord, the expression of different combinations of homeodomain and basic-helix-loop-helix transcription factors determine the identity of neural progenitors. Shh promotes the expression of some of these molecules (Class II) and represses the expression of others (Class I). Cross-repression between both classes defines ventral spinal cord domains that will generate specific types of neurons.[50-51] Knock-out mice for *shh* neither develop FP nor most ventral neuronal types, including V2 interneurons.[9] *Smo* is essential for all Hh signalling, and its loss-of-function generates a more severe phenotype, where failure in the specification of ventral cell types is more dorsally extended. The differences with *shh* mutants most likely reflect a normal contribution of Ihh signalling from the underlying gut endoderm.[52]

The three known mediators of Hh signalling are expressed in partially overlapping domains in the neural epithelium. Although their patterns are arranged in gradients with more or less widespread distributions, Gli1 is predominantly ventral, Gli3 dorsal, and Gli2 intermediate and dorsal. In mouse, Gli2 is essential for FP formation and is required, together with Gli1, for V3 development.[9] In embryos lacking all Gli function the FP and V3 interneurons do not arise. Strikingly, these mutants develop MN and V0 to V2 interneurons, but their distribution is totally disorganised. Thus, Hh signalling is essential in mouse for the specification of FP and the most ventral interneurons. Although not necessary for the induction of MN and the remaining interneurons, it regulates their spatial pattern. Transcription factors other than Gli may be responsible for inducing differentiation of some MN and V0 to V2 interneurons.[53]

Retinoids are good candidates for regulating the expression of such molecules,[51,54] and Shh signalling may be necessary for the selective survival and expansion of precursor pools.[9]

Shh As a Mitotic and Antiapoptotic Agent

The development of the CNS depends on the precise coordination of growth and patterning mechanisms. Although the latter are becoming well understood, less is known about the factors that govern the shape and size of the CNS. Recent studies indicate that Shh is involved in the control of growth and cell survival during early and late stages of development, providing cues for size and shape. Indeed, in 1950 it was already shown that the embryonic chick brain collapses if the notochord and anterior hindbrain are separated from the neuroepithelium. At first glance, these results were attributed to an "experimental overgrowth". However, although more cells were in mitosis because their cell cycle was longer, there was net cell loss. It was concluded that the notochord normally secretes a trophic factor important for the expansion of the brain vesicles. Recently, it was shown that when the notochord is transiently displaced from the midbrain FP, the brain vesicles also collapse and fold abnormally. Although patterning and differentiation is not impaired, proliferation decreases and apoptosis increases in the midbrain. This is explained by the reduction of Shh levels in the notochord and the FP, since an implant of Shh-secreting cells in the ventral midbrain reverts the effect, and the normal midbrain expansion is retarded by cyclopamine, an inhibitor of the Shh pathway.[55-56] In addition, the injection of antibodies against Shh into the chick cranial mesenchyme inhibits proliferation in the neural tube and induces massive apoptosis in cranial neural tube and neural crest.[57] This evidence supports the idea that the ventral midline of the neural tube, by secreting Shh, is involved in the process of three-dimensional shaping during the early growth of the brain by controlling proliferation and cell survival.

The anti-apoptotic role of Shh is also crucial for the development of more caudal regions of the CNS. Programmed cell death in the posterior neural tube of *shh* knock-out mice is restricted to discrete ventral and dorsal regions and occurs between E9.5 and E10.5.[58] When chick embryos are deprived of midline structures by ablation of zone c of Hensen´s node, the neural tube posterior to the excised zone develops but it is completely dorsalised and displays massive cell death after 20 h of operation. However, apoptosis is prevented when a graft of midline cells (notochord or FP) or a Shh source is provided.[16,23] These results strengthen the idea that Shh secreted by midline cells, is absolutely required to keep the neural tube alive. However, it remains to be elucidated whether the apoptosis produced by the absence of midline structures can be entirely homologated to the effects produced by removing Shh signalling. Programmed cell death has the role of sculpting the shape and size of organs. The built-in suicide program, first demonstrated in *Caenorhabditis elengans,*[59] can be seen as a default condition that must be modulated to attain the correct form and shape of the neural tube, and Shh signalling has a crucial role in this balance. In fact it was recently determined that Ptc has a proapoptotic role, which is prevented by binding of Shh.[60]

Several findings from *Xenopus* embryos indicate that Shh signalling is involved in a balance between neuronal differentiation and the control of cell number, and this balance receives the input of retinoid signalling. Overexpression of *shh* in frog embryos inhibits primary neurogenesis in the spinal cord and thickens the neural plate but later, an increase of secondary motorneurons is evident. The expansion of the neural plate was attributed to an increase in proliferation. On the other hand, when *shh* was knocked-down, primary neurogenesis was enhanced, and the absence of midline signalling impaired the normal division of the brain into two hemispheres, which resulted in diverse grades of cyclopia (Fig. 1E). Retinoids inhibit *shh* expression and enhance primary neurogenesis. To explain the opposite effects on primary and secondary neurogenesis and the counterbalancing activity of retinoids, it was proposed that Shh withdraws neural precursors from premature differentiation by retinoid signalling, holding their proliferative state and reserving them for subsequent waves of differentiation.[38,61-62] These results are consistent with findings from mouse embryos. Ectopic expression of *shh* in the

dorsal neural tube of transgenic mice induces proliferation of neural precursors and inhibits their differentiation.[14] In *shh* knock-out mice the telencephalon is 90% smaller than normal and consists of a single fused vesicle, strongly dorsalised; ventral and dorsal diencephalic structures are also reduced. This dramatic phenotype is due to the disruption of brain proliferation and to increased apoptosis.[30]

Shh is expressed in a layer-specific manner in the perinatal mouse neocortex and tectum, while *gli* transcripts are found in proliferative zones. *Shh* is required as a mitogen after stage E12 in the superficial layer of the tectum and neocortex (layer V) and also in ventricular and subventricular zones, where g*li* genes are expressed. This resembles the situation in the cerebellum, where Shh secreted by the Purkinje neurons induces proliferation of the granular layer. It is also similar to the mechanism in the hippocampus, where secreted Shh from cells in the hilus of the dentate gyrus induces proliferation of granular and septal cells.[63-65] Therefore, Shh signalling associated with proliferative regions could be part of a general mechanism of control of the cell number by regulating cell cycle and cell death.

Besides its role during CNS development, a growing line of evidence points to a crucial role of Shh signalling in the maintenance of postnatal and adult telencephalic stem cell niches. For example, the adult rat hippocampus expresses high levels of *ptc,* and when exposed to an adeno-associated viral vector delivering *shh* cDNA, a potent mitogenic effect is observed. Neural progenitors isolated from this region and cultured with *Shh* proliferate, retaining their multipotency.[66] Conditional null alleles of *shh* and *smo* display increased apoptosis of neural progenitors in the postnatal subventricular zone and reduced proliferation in the gyrus dentate.[67]

Therefore, Shh has been consolidated as an anti-apoptotic and mitogenic factor that controls growth and shape during the development of the CNS and it is also present in adult neurogenic niches, where the complex architecture requires premature differentiation to be inhibited on behalf of remodelling and plasticity.[68-69]

Closing the Idea

The midline structure composed by the FP and the notochord is formed by a crucial binary switch executed by Delta-1/Notch/hairy-2, which controls the distribution of cell fates in the organiser, and Shh contributes to refine the shape and size of both structures. In turn, their size provide the basis for the number of cells that secrete Shh, whose diffusion influences (1) the shape and size of the neural plate, by controlling cell number; (2) the correct patterning of the neural tube. Therefore, building of the midline is a crucial part of the program by which the organiser commands the distribution of signals and cell fates to insure the proper organising activity defined by Hilde Mangold and Hans Spemann in 1924.[33]

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