The emergent design of the neural tube: prepattern, SHH morphogen and GLI code

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The Sonic hedgehog (Shh) pathway plays an important role in the development of many tissues and organs. The secreted ligand Shh has been shown to act as a mitogen, morphogen and survival factor in different contexts whereas the three Gli transcription factors act as Shh mediators in a context-dependent combinatorial fashion. The common wisdom has been that Gli protein function is subject to Shh signaling. One can ask how Gli proteins act and what the nature of Shh signaling during CNS dorsal–ventral patterning is. Is it possible that Hedgehog signals are only one of several ways to regulate Gli activity? Moreover, in light of the partial rescue of the neural tube phenotype of *Shh* or *Smoothened* mutant embryos in *Shh*$^{-/-}$;*Gli3*$^{-/-}$, *Smoothened*$^{-/-}$;*Gli3*$^{-/-}$, and *Shh*$^{-/-}$;*Rab23*$^{-/-}$ double null embryos, one can consider the roles that the Shh–Gli pathway may have taken to orchestrate congruent prepattern and growth, and the importance of creating the correct number of precursors in patterning mechanisms.

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Abbreviations

BMP	bone morphogenetic protein
CNS	central nervous system
D–V	dorsal–ventral
FGF	fibroblast growth factor
Hh	Hedgehog
IGF	insulin-like growth factor
Ihh	Indian hedgehog
Ptch1	Patched1
Shh	Sonic hedgehog
Smo	Smoothened

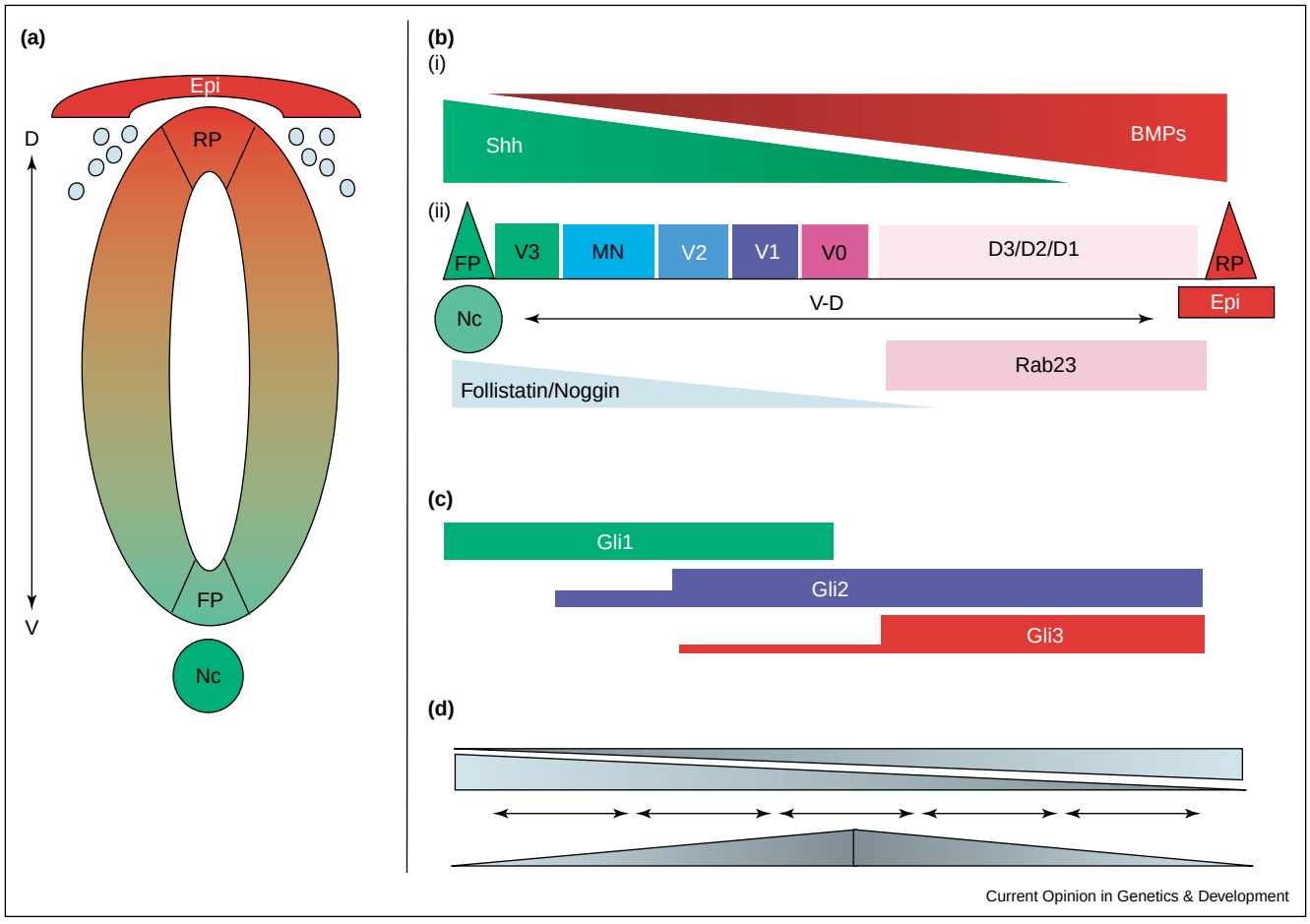
Introduction

Within the central nervous system (CNS), the development of the early vertebrate ventral neural tube [1] and of the later dorsal brain [2] depends on Sonic hedgehog (Shh) signaling. Shh has been shown to have many functions. In the early neural tube, it is proposed to act as a morphogen to specify ventral fates (e.g. see [3–5,6•]; Figure 1a,b). By contrast, in late brain development Shh seems to act as a mitogen on progenitors of the cerebellum, tectum, neocortex and hippocampus [7–10,11•]. Here, we review some basic aspects of Shh and Gli function to discuss how Shh acts as a neural tube morphogen responsible for combinatorial Gli function and whether it is only one of several informational inputs that create a morphogenetic Gli code. We also address the existence and integration of Shh-independent patterning mechanisms.

A multitude of experimental results demonstrates a clear and essential participation of Hedgehog (Hh) signals in a great variety of processes in animal development [12]. Variations in the site, strength or timing of these signals may underlie evolutionary and ontogenetic changes in the size, shape and location of many cell groups and organs. Hh proteins, through the regulation of Gli proteins in precursors, may endow the organism with a redundant set of morphogenetic possibilities allowing the species and the individual a certain degree of morphological plasticity and adaptability [2]. In the early neural tube, several findings support the idea that Shh acts as a morphogen: first, the graded distribution of the Shh ligand [13]; second, its concentration-dependent effects on cells of intermediate neural plate explants [14,15]; third, the position-dependent re-specification of pattern in the neural tube after grafting an ectopic notochord or supplying an ectopic source of Shh [5,16,17]; fourth, the direct action of Shh ligand on Smoothened (Smo) expressing cells at a distance [4,6•,18]; fifth, the loss of ventral cell types in the *Shh*$^{-/-}$ mouse [19]; and sixth, the re-specification of the neural tube in *Patched1*$^{-/-}$ mice [20].

Secreted Hh ligands act extracellularly via the Patched1 (Ptch1) and Smo transmembrane proteins to activate an intracellular information transfer cascade [21] that is interpreted and decoded by the action of three zinc finger transcription factors: the Gli proteins [12,22]. An active pathway can be detected by the elevated levels of *Gli1* and *Ptch1* transcripts. Gli proteins appear to be obligatory final mediators of Hh signals to activate the expression of Hh-responsive genes, but Gli proteins can also either respond to or modify information inputs unrelated to Hhs. For example, Gli2 and Gli3, but not Gli1, are implicated in FGF signaling in mesodermal development of the early frog embryo [23]. In the neural tube, the antagonistic relationship of Shh and BMP signaling [24] could involve a physical interaction between Smad and Gli proteins [25]. Gli factors, in turn, activate other signaling cascades as well as intracellular information transfer. For example, IGF signaling and batteries of

Figure 1

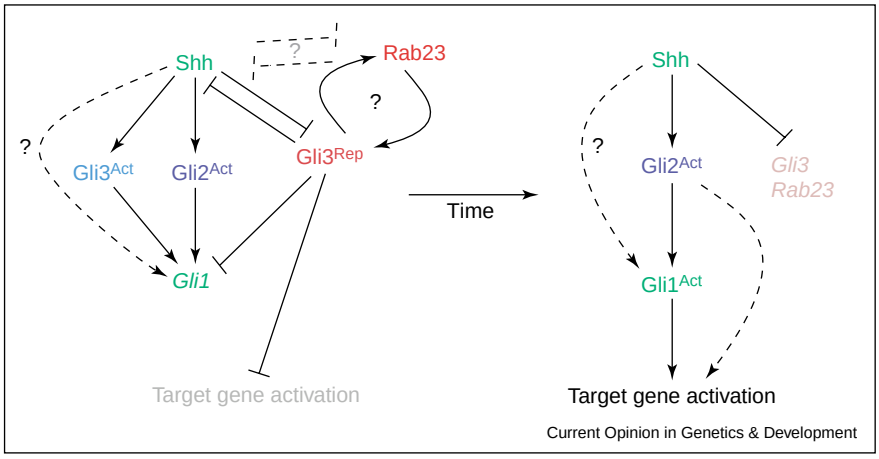


Dorsoventral pattern of the neural tube. **(a)** Schematic representation of a cross section through an archetypal embryonic vertebrate neural tube showing the position of polar cell groups, the floor plate (FP; green) ventrally and the roof plate (RP; red) dorsally. The epidermis (Epi; red), covering the dorsal neural tube and the notochord (Nc; green), underlying the ventral aspect are also shown. D–V pattern is suggested by the graded color change (red to green). Neural crest emigrating from the dorsal neural tube is shown in light blue. **(b)** D–V cell pattern and signaling function. (i) Diagram of the graded patterning functions of Shh signaling ventrally (green) and BMP signaling (red). Other signaling molecules (e.g. Wnts and Nodal) are not shown. (ii) Schematic representation of the D–V pattern of the neural tube seen after bisecting the neural tube and flattening one side. Cell types are from ventral to dorsal: floor plate (FP), V3, MN, V2, V1, V0 and D3–D1 neurons, and roof plate. A possible V–D gradient of BMP antagonists, follistatin and noggin, is included (light blue), and the location of Rab23 expression denoted dorsally (light purple). **(c)** Approximate expression domains of the three *Gli* genes along the D–V axis of the neural tube. Note that the domains and boundaries change with time [40,41]. **(d)** Speculative possibilities for the source of D–V prepattern: long-range graded functions from neural or non-neural cells, local interactions and relay mechanisms and the creation of a mid neural tube organizer boundary and center.

Wnt genes are targets of Shh–Gli function [26,27] and Shh regulates *Cyclin D* and *N-Myc* [28–30]. In the early brain, Shh also seems to control FGF signaling to induce cell proliferation [31,32]. Although the informational inputs that regulate Gli function and the targets of these transcription factors must be defined further, in one instance, Gli2 orchestrates ventroposterior mesodermal development by regulating at least three different types of targets. These are genes involved in morphogenesis (*Wnts*), tissue specification (*Brachyury*) and positional information (*Xhox3*) [23]. In another instance, Gli proteins induce the neurogenic basic helix-loop-helix cascade during neuronal development in the neural plate [33]. Moreover, in the limb, Gli3 represses *dHand* and *Gremlin* to contribute to the elaboration of digit identity [34•,35•]. In the neural tube, Gli proteins appear to regulate a set of Shh-responsive homeobox genes that elaborate specific ventral cell fates [36,37•]. These and other results are consistent with the proposal that Gli proteins are critical mediators of Hh signals but also integrators of other informational inputs as well as regulators of secondary signaling and that the exact outcome of *Gli* function and their interactions are context-dependent [38,39].

The three Gli genes are expressed in partially overlapping domains in the neural tube (Figure 1c) but in the

Figure 2



Schematic representation of the dynamic interaction between (left) early and (right) late responses to Shh signaling in the ventral neural tube. Note the proposed dual role of Gli3. How and if Rab23 affects Gli function is unknown. See main text for details. Dashed symbols represent possible interactions.

early neural plate, their expression is more widespread [40,41]. All three Gli proteins have activating function and only Gli2 and Gli3 appear to harbor potent repressor activity, especially as proteolyzed products lacking the C termini. As in *Drosophila* [42], C-terminally deleted Gli proteins have dominant-negative function [43–46]. However, each Gli seems to be regulated differently. For example, Gli1 may be a constitutive strong activator of transcription [40,47,48] whereas Gli2 activator function appears to be enhanced by Hh signaling [49]. In addition, Hh signaling inhibits the formation of the Gli3 repressor form but apparently not that of Gli2 [43–46,49] (Figure 2).

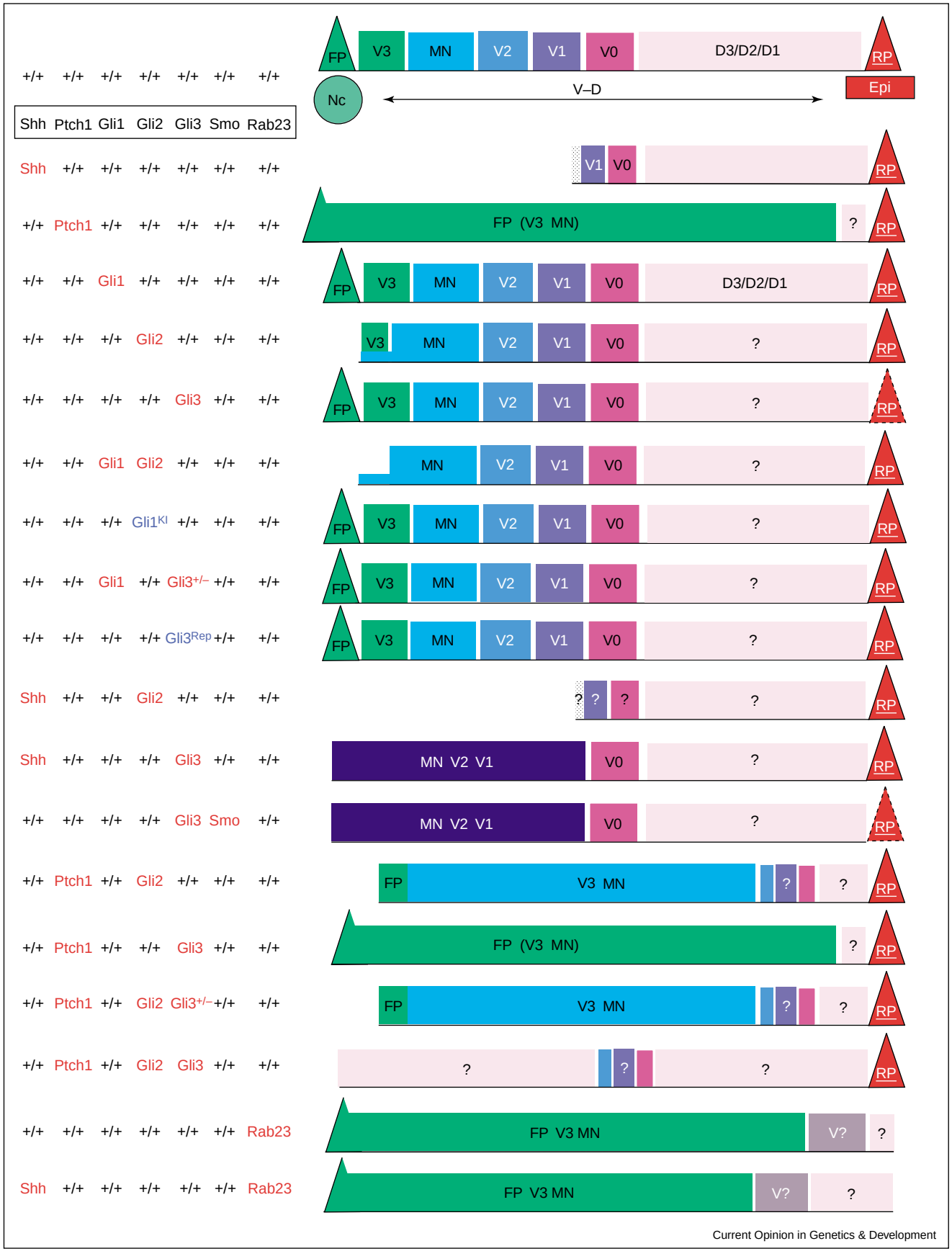
An initial model for combinatorial Gli activity [38] suggested a positive function of Gli1, an antagonistic relationship for Gli3 and a dual role for Gli2. Despite the strong activity of Gli1, its mimicry of Shh signaling and its loyal expression in cells receiving Shh signals, it may not be a universal initial Hh mediator. Pre-existent Gli2 and Gli3 proteins have been proposed to mediate initial Hh signaling and to regulate Gli1 [44,45]. Shh–Gli1 may thus have a dual relationship with Gli3/2, with these proteins (possibly in a full-length form) mediating the initial activation or enhancement of *Gli1* by Shh and later on (maybe as truncated repressors) acting to inhibit positive Gli1 function (Figure 2). Here, Gli3 would act as a delayed intrinsic antagonist. Although the exact function of each Gli protein varies in different species [50,51], loss of Gli1 in mice is compensated by other Gli proteins [52,53], loss of Gli3 does not appear to lead to loss of dorsal cell types in the spinal cord [37•,54] although intermediate patterning is abnormal [37•], and loss of Gli2 affects only few ventral cell types, most notably the floor plate [55–57]. If Gli proteins are required for neural tube patterning, then there must be a relatively large degree of redundancy and compensation of Gli function, at least Gli2 for Gli3 dorsally and Gli2 for Gli1 ventrally. However, even though it has been done in a heterologous system, Gli1 and Gli2 preferentially regulate different Cubitus interruptus targets in transgenic flies [49], and they seem to regulate different targets in the neural tube [58•] a result that seems at odds with the rescue of the *Gli2* mutant by a *Gli1* knock-in into the *Gli2* locus [59•].

With these results and considerations in mind, the finding that the severe cyclopia and the loss of ventral neural cell types, including motor neurons, seen in mice lacking Shh function [19] is partly rescued by the concomitant loss of Gli3 [60], Smo [6•] or Rab23 (a vesicle transport family protein [61–63]) is remarkable (Figure 3). These intriguing results prompt the evaluation of several issues. First, the phenotype of double nulls indicates that Gli3 and Rab23 function normally to antagonize that of Shh signaling and vice versa. Second, in the absence of Gli3 and Shh, or Rab23 and Shh, the differentiation of most ventral cell types occurs, but their distribution and the size of the neural tube is abnormal. Third, Gli1/2 are not expected to function normally in *Shh* null mice so that the double *Shh*;*Gli3* null might have little positive Gli activity. Is there then dorsal–ventral (D–V) prepattern independent of Shh–Gli signaling? Fourth, how are putative prepattern mechanisms and Shh–Gli function integrated to create consistent pattern and size? Fifth, what is the nature of such mechanisms?

Functional antagonisms and the creation of graded activities

The *Shh*;*Gli3* double mutant phenotype (Figure 3) indicates that the function of these genes antagonize each

Figure 3


Shh Ptch1 Gli1 Gli2 Gli3 Smo Rab23
Nc
V–D
FP
V3
MN
V2
V1
V0
D3/D2/D1
RP
Epi
FP (V3 MN)
?
MN V2 V1
V3 MN
Gli1KI
Gli3+/–
Gli3Rep
FP V3 MN
V?
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other. This is consistent with the antagonism of Shh/Gli1 and Gli3 originally described in frog embryos [40,50] and with the ability of Gli3 to encode a potent repressor of positive Gli function [43–45]. Gli3 also has a positive function [23,27,33,45] and many abnormalities detected in the Gli3 null mouse outside of the CNS are not rescued in the *Shh*;*Gli3* null [19,35•]. The loss of both positive and negative activities in the *Gli3* (Xt^J) mutant could contribute to its imperfect rescue of the Shh null phenotype. However, it is the repressor function that appears to participate in the patterning of the intermediate neural tube as homozygous mice for a C-terminally truncated mutation yielding a repressor-like *Gli3* gene is reported to result in normal CNS development but with other organ abnormalities [37•,64]. Because Shh acts in a concentration-dependent manner, and the rescue phenotype in double mutants is graded, with an intermediate result seen in $Shh^{-/-}$;$Gli3^{+/-}$ animals [60], Shh may act to create a gradient of overall Gli function [38,39], likely the summation of a gradient of positive activity of Gli1/2 (and possibly Gli3) and, with opposite polarity, a gradient of negative activity of Gli3 and possibly of Gli2 as well [19,37•,38,39,65]. Here Gli2/3 may act in a dual fashion.

Shh signaling also interacts with Rab23 to establish D–V pattern. The mouse *open brain* mutation affects *Rab23* and leads to the development of neural tube that resembles that of $Ptch1^{-/-}$ mice [63]. This suggests that Shh signaling and Rab23 have an antagonistic relationship, much like *Shh* and *Gli3*. Interestingly, the graded expression of *Ptch1* in *Rab23* null embryos appears to be maintained (and possibly expanded) in *Shh*;*Rab23* double mutants [63], indicating that loss of Rab23 somehow obviates the need of the ligand Shh to create a V–D graded expression of *Ptch1*. How this is achieved and whether Rab23 affects Gli3 function is not known.

The problem of size

Shh null embryos lack ventral cell types, including floor plate, motor neurons, V3 and frequently V2 neurons. In *Shh*;*Gli3* nulls, there is ventral cell type differentiation with V2 and motor neurons present [54], although there are fewer cells and their positions are abnormal. Therefore, the complete loss of *Gli3* does not fully rescue the *Shh* null phenotype. Indeed, there is also no floor plate differentiation in the double mutants, a result that may highlight the requirement of Gli2 in this process [53]. However, on the one hand, this partial rescue implicates Gli3 as a negative regulator of cell number — perhaps as an anti-mitogenic or pre-apoptotic factor — on the other, the abnormal ventral cell number in the *Shh*;*Gli3* double mutants implicates Shh-driven positive function in the elaboration of the correct number of cells. Gli1 and Gli2 may mediate this effect, but the size of the neural tube in $Gli2^{-/-}$ or $Gli1^{-/-}$;$Gli2^{-/-}$ animals is nearly normal [56].

The size of the neural tube is reduced drastically in *Shh* null mice, being restored to that of wild-type littermates in the absence of *Gli3*. The smaller size of the neural tube is a clear sign of loss of ventral differentiation seen in the original notochord removal experiments — and thus removal of the initial source of Shh signals for CNS patterning [14,66]. A smaller neural tube also seems to be accompanied by an increase in cell death [16,67], blurring the boundaries of the functions of Shh signaling in morphogenetic patterning, survival and cell proliferation. These effects could all take place simultaneously on precursor cells as Shh–Gli affects the precursors of ventral neuronal types [68] and the precursors of V0–V1 and motor neurons are restored in the *Shh*;*Gli3* and *Smo*;*Gli3* double mutants [6•,60]. This indicates that size regulation affects the survival, maintenance or expansion of precursor pools. Interestingly, cells in the dorsal spinal cord, like those in the cerebellum [7–9,20] and neocortex [10], also respond to either increased or ectopic Shh signaling by hyper-proliferating [69]. Whether Shh–Gli function acts on stem cells and/or committed precursors remains to be determined.

Prepattern, Shh–Gli-independent mechanisms and the Gli code

If Shh induces the positive activity of Gli1 and Gli2 and concomitantly the inhibition of Gli3 repressor activity, the double *Shh*;*Gli3* mutant will lack positive Gli function, thus perhaps explaining the fact that the rescue of the *Shh* null phenotype is only partial. (In *Shh* nulls, *Gli1* is almost absent.) The embryonic neural tube, however, seems to be receptive to other sources of Hh signals. This is highlighted by the more severe phenotype of *Smo* null mice versus *Shh* nulls [6•]. Double *Ihh* (*Indian hedgehog*);*Shh* nulls phenocopy the *Smo* null phenotype. *Ihh*, one of the three mouse Hh genes, is expressed in the

(Figure 3 Legend) Schematic representation of the D–V pattern of the neural tube, as shown in Figure 1b, in the different genetic backgrounds described in the right column (+/+ refers to wild type; +/− denotes heterozygotes). Genes in red denote null (−/−) mutations. Genes in blue represent the Gli1 knock-in into the *Gli2* locus ($Gli21^{KI}$) or the deletion of the C terminus of Gli3 yielding a Gli3 repressor like constitutive function (Gli3•699 [64]). The status of dorsal cell types has not been generally assessed as is denoted by a question mark. *Gli3* and *Opb* mutants have exencephaly ([61–63,81,82]. The status of the roof plate in the former is not known as is denoted by a triangle with broken line. Overlap of cell types in different mutants is shown by overlapping colors or a new color/fill-in pattern with multiple neuronal types. The status of floor-plate development in the Gli2 null and Gli2;Ptch1 double null embryos is controversial (see [56,57,58•,59•]). Note that the requirements for Gli2 and Gli3 vary along the neuraxis and results shown in the figure were obtained at the anterior thoracic level (see [58•]). Sizes of the neural tube and domains are all approximate. For details, see the text and the following primary references from which the data is derived. Shh [60], Ptch1 [20,58•,59•], Gli1 [52,59•], Gli2 [56,57,58•], Gli1;Gli2 [52], Gli21KI [59•], Gli1;Gli3 [52], Gli3Rep (Gli3•699) [37•], Shh;Gli2 [53], Ptch1;Gli2 [58•,59•], Ptch1;Gli3 (mentioned in [58•]), Ptch1;Gli2;Gli3 [58•], Gli3 and Shh;Gli3 [37•,60], Smo;Gli3 [6•], Rab23 [61,62], Shh;Rab23 [63].

developing gut tube and early in embryogenesis the gut tube is in close proximity to the neural tube. This suggests that in the neural tube of the double *Shh*;*Gli3* mutant there is a low level of Hh signaling and thus of Gli1/2 function. The low level of Gli1/2 function, together with the loss of the opposite Gli3 repressor gradient in the *Shh*;*Gli3* double null, could result in the development of partial ventral pattern. Nevertheless, the ventral pattern in *Shh*;*Gli3* nulls is similar to that of *Smo*;*Gli3* double mutants [6•,70], indicating that in the complete absence of Hh signaling (in *Smo* nulls) and thus likely of positive Gli1/2 function, loss of *Gli3* partially rescues ventral pattern. These results raise the possibility that in the possible absence of Hh and Gli3 activity, and likely of activating Gli1/2 function, the neural tube obtains partial D–V patterning, revealing perhaps Shh–Gli-independent D–V prepattern. For example, some precursors may attain a pre-identity through various mechanisms and Shh signaling may act on most of these initial identities to modify and orchestrate them. Perhaps the cell types that remain in *Shh*;*Gli3* double nulls reflect some of these initial events that may be left untouched. Alternatively, Shh signaling may suppress prepattern and impose novel patterning. One of the critical functions that Shh signaling may have developed, in some but not all cases, is the sorting of like cells, possibly through the regulation of cell affinities [71]. This would allow the formation of neuronal pools, in distinction, for example, to the scattering of spinal oligodendrocytes derived from tightly localized ventral precursors [72].

A role for Gli proteins could still be invoked in the absence of Hh signalling. If Gli2, for example, were to respond to informational inputs in addition to Hhs, its maintained expression in *Gli3* null and *Shh* null mice ([4]; V Palma, A Ruiz i Altaba, unpublished data) could account for a central role of Gli proteins in both Hh-dependent and Hh-independent scenarios. However, *Gli2* mutants partially rescue the *Ptch1* phenotype [57], suggesting that it is required for Shh signal mediation but ventral neuronal pattern is not affected in *Gli1* and not severely affected in *Gli2* or *Gli1*;*Gli2* double nulls [52]. Thus, either Gli3 takes an unexpected, positive role in the ventral neural tube in these mutant mice, or there is a non-Gli mediator of Shh signals. It remains possible that dorsal BMPs or Wnts activate the dorsal expression or affect the activity of Gli2 and Gli3 [65,73]. The fact that BMPs can maintain expression of *Gli3* in dorsal neural tube explants [65] or that presomitic mesoderm cultured on Wnt-expressing cells results in *Gli2/3* expression in the resulting somites [73] does not allow one yet to determine if their expression is regulated by such signals or whether it is just a marker of appropriately formed or maintained tissues (the dorsal neural tube and the somites normally express Gli2/3). If Gli proteins do respond to multiple signals in the CNS, they could act as critical integrators of multiple patterning informational inputs, thereby dictating the fate of neural tube precursors [2,22]. In this case, a Gli code would be established according to the integration of all incoming patterning information that affects them.

The partial rescue of the *Shh* null phenotype by loss of *Rab23* suggests an interaction between Shh signaling and Rab23 function. As Rab family proteins are involved in vesicle trafficking, Rab23 could affect some intracellular aspects of Shh signaling. Analyses of chimeras, however, indicate that it acts both non-cell-autonomously and cell-autonomously [62]. The persistence of a graded expression of *Ptch1* in *Rab23* null and in *Rab23*;*Shh* null embryos raises the possibility that in the latter there is active Shh–Gli pathway activity in the absence of Shh. Here, Rab23 could affect Ptch1 directly, which would then translate into an effect on Smo and pathway activity. Another possibility is that Rab23 acts or affects a Shh-independent patterning activity. For example, Rab23 and Gli3 could affect each other's function or Rab23 may respond or affect BMP or Wnt signaling.

Requirement of positive Gli function and integration of patterning mechanisms

Widespread expression of Gli3 or Gli2 repressors in the frog neural plate abolishes neuronal differentiation [33] and widespread expression of a Gli3 repressor in the chick neural tube abolishes ventral cell differentiation [55,65]. These results alone suggest that positive Gli function (or the regulated expression of Gli targets) is required for neuronal development and ventral neural tube cell differentiation. It remains to be tested whether the activator and repressor forms of Gli proteins act on the same targets. In flies, the activator and repressor forms of Cubitus interruptus can regulate different targets [74]. These experiments, however, were performed in the context of normal Hh signaling. If the action of an independent patterning mechanism were inhibited or modified by Shh signaling, thus supplanting the role (and primacy) of the former, the results could be interpreted to mean that as long as such a patterning mechanism is inhibited or altered, the function of a combinatorial Gli code is required to serve, mediate and interpret Hh signals and establish pattern. It remains to be seen what degree of pattern develops in the neural tube of mice lacking all Gli function with and without Shh signaling. If *Smo*;*Gli3* double nulls, having no canonical Shh signaling activity, were to lack all positive Gli function, this would mean that the specification of ventral cell types (and thus of their precursors) can take place without Gli proteins. Nevertheless, because these ventral cell types are localized randomly in the double null, Gli function might orchestrate and organize pre-existent pattern.

How Shh signaling inhibits or modifies pre-existent D–V patterning mechanisms is not known. One possibility is that there is a Gli-independent mechanism for pathway

suppression or alteration at play. In this sense, it is notable that Ptch1 has been proposed to act on cyclin B directly [75], raising the possibility that Hh action may alter activities of Ptch1/2 or even Smo, which are unrelated to the canonical pathway and Gli function. Alternatively, because Shh-independent patterning is revealed only in the concomitant absence of Gli3 [60], we could propose that Gli3 repressor function may have been co-opted for the suppression or alteration of such independent mechanisms, with positive Shh–Gli1/2 (and possibly Gli3) function imposed on it to create pattern. In this model, positive Gli function may thus orchestrate spatial and temporal order to independent patterning mechanisms, indicating that Shh–Gli function behaves as an organizer mechanism, which reframes pre-existing mechanisms but could be also subject to their availability. This model is consistent with the loss of neuronal pattern after misexpression of a synthetic Gli3 repressor [33,37•,65] and with the modification of the result of Shh signaling by BMP antagonists [24].

Possible nature of Shh-independent dorsal–ventral patterning mechanisms: the bases of prepattern

If Hh and/or Gli proteins indeed suppress or modify latent patterning mechanisms, and by doing so impose further evolutionary plasticity, what is the nature of such mechanisms? The answer to this question is not known (Figure 1d) and we can only speculate that patterning might occur through one of the usual suspects involved in intercellular signaling and patterning including the Wnt and BMP pathways (Figure 1b). But one cannot completely rule out possible effects from other molecules or properties (e.g. calcium or even oxygen gradients). Both Wnts and BMPs are expressed in different locations of the developing neural tube and there is also localized expression of inhibitory molecules. For example, ventral expression of BMP inhibitors, such as follistatin and noggin [24,76], may result in a D–V gradient of BMP function that could be instructive. Interestingly, BMP and Shh signaling inhibit each other [77], BMP inhibitors, modulate the effects of Shh signaling [24] and Smads and Gli proteins can interact [25], suggesting an interplay between these two pathways, as well as a potential site for informational integration. Another possible pathway may be that initiated by retinoic acid, which is involved in early neural-plate patterning [78,79] and the later elaboration of ventral interneuron fates [80].

Conclusions

Taken together, the issues raised in this discussion suggest an interesting role of Shh–Gli signaling in the elaboration of novel ontogenic and evolutionary processes and highlight the fact that we are only beginning to understand how this pathway has been deployed during the development of the embryo and during the formation of many organs. Perhaps the blur in the distinction of the survival, proliferative and morphogenetic roles of Shh signaling is somewhat meaningful. The morphogenetic action of Hh does not just affect ventral cell differentiation in the neural tube; it also induces the production of the correct numbers of precursors that can then respond to (other) appropriate patterning signals. Shh signaling could thus possibly function to ensure that independent mechanisms act on the correct number of precursors, resulting in appropriate pattern and size. Indeed, pattern-formation and differentiation mechanisms must account not only for appropriate cell type determination but also for the varying and species-specific sizes of cell groups and organs, and inversely, size and form of the tissues affect the outcome of pattern-formation and differentiation mechanisms. The neural tube has, therefore, not been designed as such. Rather, and much as our larger self, it appears to be the emergent result of a multitude of intertwined layers of information that are re-enacted each time an embryo develops, the origins of which lie in its evolutionary history.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Jessell TM: **Neuronal specification in the spinal cord: inductive signals and transcriptional codes**. *Nat Rev Genet* 2000, **1**:20-29.

2. Ruiz i Altaba A, Palma V, Dahmane N: **Hedgehog-Gli signaling and the growth of the brain**. *Nat Rev Neurosci* 2002, **3**:24-33.

3. Hynes M, Ye W, Wang K, Stone D, Murone M, Sauvage F, Rosenthal A: **The seven-transmembrane receptor smoothened cell-autonomously induces multiple ventral cell types**. *Nat Neurosci* 2000, **3**:41-46.

4. Briscoe J, Chen Y, Jessell TM, Struhl G: **A hedgehog-insensitive form of patched provides evidence for direct long-range morphogen activity of sonic hedgehog in the neural tube**. *Mol Cell* 2001, **7**:1279-1291.

5. Agarwala S, Sanders TA, Ragsdale CW: **Sonic hedgehog control of size and shape in midbrain pattern formation**. *Science* 2001, **291**:2147-2150.

6. • Wijgerde M, McMahon JA, Rule M, McMahon AP: **A direct requirement for Hedgehog signaling for normal specification of all ventral progenitor domains in the presumptive mammalian spinal cord**. *Genes Dev* 2002, **16**:2849-2864.
Analyses of *Smo* mutant cells and the rescue by loss of Gli3.

7. Dahmane N, Ruiz i Altaba A: **Sonic hedgehog regulates the growth and patterning of the cerebellum**. *Development* 1999, **126**:3089-3100.

8. Wechsler-Reya RJ, Scott MP: **Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog**. *Neuron* 1999, **22**:103-114.

9. Wallace VA: **Purkinje-cell-derived Sonic hedgehog regulates granule neuron precursor cell proliferation in the developing mouse cerebellum**. *Curr Biol* 1999, **9**:445-448.

10. Dahmane N, Sánchez P, Gitton Y, Palma V, Sun T, Beyna M, Weiner H, Ruiz i Altaba A: **The Sonic Hedgehog-Gli pathway regulates dorsal brain growth and tumorigenesis**. *Development* 2001, **128**:5201-5212.

11. • Lai K, Kaspar BK, Gage FH, Schaffer DV: **Sonic hedgehog regulates adult neural progenitor proliferation *in vitro* and *in vivo***. *Nat Neurosci* 2003, **6**:21-27.
Demonstration of the mitogenic activity of Shh on hippocampal progenitors.

12. Ingham PW, McMahon AP: **Hedgehog signaling in animal development: paradigms and principles**. *Genes Dev* 2001, **15**:3059-3087.

13. Gritli-Linde A, Lewis P, McMahon AP, Linde A: **The whereabouts of a morphogen: direct evidence for short- and graded long-range activity of hedgehog signaling peptides**. *Dev Biol* 2001, **236**:364-386.

14. Yamada T, Pfaff SL, Edlund T, Jessell TM: **Control of cell pattern in the neural tube: motor neuron induction by diffusible factors from notochord and floor plate**. *Cell* 1993, **73**:673-786.

15. Briscoe J, Sussel L, Serup P, Hartigan-O'Connor D, Jessell TM, Rubenstein JL, Ericson J: **Homeobox gene Nkx2.2 and specification of neuronal identity by graded Sonic hedgehog signaling**. *Nature* 1999, **398**:622-627.

16. Van Straaten HW, Hekking JW, Beursgens JP, Terwindt-Rouwenhorst E, Drukker J: **Effect of the notochord on proliferation and differentiation in the neural tube of the chick embryo**. *Development* 1989, **107**:793-803.

17. Yamada T, Placzek M, Tanaka H, Dodd J, Jessell TM: **Control of cell pattern in the developing nervous system: polarizing activity of the floor plate and notochord**. *Cell* 1991, **64**:635-647.

18. Hynes M, Ye W, Wang K, Stone D, Murone M, Sauvage F, Rosenthal A: **The seven-transmembrane receptor smoothened cell-autonomously induces multiple ventral cell types**. *Nat Neurosci* 2000, **3**:41-46.

19. Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, Westphal H, Beachy PA: **Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function**. *Nature* 1996, **383**:407-413.

20. Goodrich LV, Milenkovic L, Higgins KM, Scott MP: **Altered neural cell fates and medulloblastoma in mouse patched mutants**. *Science* 1997, **277**:1109-1113.

21. Ruiz i Altaba A: **The works of GLI and the power of hedgehog**. *Nat Cell Biol* 1999, **1**:E147-E148.

22. Ruiz i Altaba A: **Gli proteins and Hedgehog signaling: development and cancer**. *Trends Genet* 1999, **15**:418-425.

23. Brewster R, Mullor JL, Ruiz i Altaba A: **Gli2 functions in FGF signaling during antero-posterior patterning**. *Development* 2000, **127**:4395-4405.

24. Liem KF, Jessell TM, Briscoe J: **Regulation of the neural patterning activity of sonic hedgehog by secreted BMP inhibitors expressed by notochord and somites**. *Development* 2000, **127**:4855-4866.

25. Liu A, Joyner AL, Turnbull DH: **Alteration of limb and brain patterning in early mouse embryos by ultrasound-guided injection of Shh-expressing cells**. *Mech Dev* 1998, **75**:1007-1015.

26. Hahn H, Wojnowski L, Specht K, Kappler R, Calzada-Wack J, Potter D, Zimmer A, Muller U, Samson E, Quintanilla-Martinez L, Zimmer A: **Patched target Igf2 is indispensable for the formation of medulloblastoma and rhabdomyosarcoma**. *J Biol Chem* 2000, **275**:28341-28344.

27. Mullor JL, Dahmane N, Sun T, Ruiz i Altaba A: **Wnt signals are targets and mediators of Gli function**. *Curr Biol* 2001, **11**:769-773.

28. Kenney AM, Rowitch DH: **Sonic hedgehog promotes G_1 cyclin expression and sustained cell cycle progression in mammalian neuronal precursors**. *Mol Cell Biol* 2000, **20**:9055-9067.

29. Kenney AM, Cole MD, Rowitch DH: **N-myc upregulation by sonic hedgehog signaling promotes proliferation in developing cerebellar granule neuron precursors**. *Development* 2003, **30**:15-28.

30. Oliver TG, Grasfeder LL, Carroll AL, Kaiser C, Gillingham CL, Lin SM, Wickramasinghe R, Scott MP, Wechsler-Reya RJ: **Transcriptional profiling of the Sonic hedgehog response: a critical role for N-myc in proliferation of neuronal precursors**. *Proc Natl Acad Sci USA* 2003, **100**:7331-7336.

31. Ishibashi M, McMahon AP: **A sonic hedgehog-dependent signaling relay regulates growth of diencephalic and mesencephalic primordia in the early mouse embryo**. *Development* 2002, **129**:4807-4819.

32. Ohkubo Y, Chiang C, Rubenstein JL: **Coordinate regulation and synergistic actions of BMP4, SHH and FGF8 in the rostral prosencephalon regulate morphogenesis of the telencephalic and optic vesicles**. *Neuroscience* 2002, **111**:1-17.

33. Brewster R, Lee J, Ruiz i Altaba A: **Gli/Zic factors pattern the neural plate by defining domains of cell differentiation**. *Nature* 1998, **393**:579-583.

34. • te Welscher P, Zuniga A, Kuijper S, Drenth T, Goedemans HJ, Meijlink F, Zeller R: **Progression of vertebrate limb development through SHH-mediated counteraction of GLI3**. *Science* 2002, **298**:827-830.
Partial rescue of *Shh* mutant phenotype by decrease in Gli3 function in the limb.

35. • Aoto K, Nishimura T, Eto K, Motoyama J: **Mouse GLI3 regulates Fgf8 expression and apoptosis in the developing neural tube, face, and limb bud**. *Dev Biol* 2002, **251**:320-332.
Rescue of *Fgf8* expression, lost in *Shh* mutants, by the concomitant loss of Gli3.

36. Briscoe J, Ericson J: **Specification of neuronal fates in the ventral neural tube**. *Curr Opin Neurobiol* 2001, **11**:43-49.

37. • Persson M, Stamataki D, te Welscher P, Andersson E, Bose J, Ruther U, Ericson J, Briscoe J: **Dorsal-ventral patterning of the spinal cord requires Gli3 transcriptional repressor activity**. *Genes Dev* 2002, **16**:2865-2878.
Evidence that Gli3 acts as repressor in the neural tube required for ventral patterning.

38. Ruiz i Altaba A: **Catching a Gli-mpse of Hedgehog**. *Cell* 1997, **90**:193-196.

39. Ruiz i Altaba A, Palma V, Dahmane N: **Hedgehog-Gli signalling and the growth of the brain**. *Nat Rev Neurosci* 2002, **3**:24-33.

40. Lee J, Platt KA, Censullo P, Ruiz i Altaba A: **Gli1 is a target of Sonic hedgehog that induces ventral neural tube development**. *Development* 1997, **124**:2537-2552.

41. Sasaki H, Hui C, Nakafuku M, Kondoh H: **A binding site for Gli proteins is essential for HNF-3beta floor plate enhancer activity in transgenics and can respond to Shh *in vitro***. *Development* 1997, **124**:1313-1322.

42. Aza-Blanc P, Ramirez-Weber FA, Laget MP, Schwartz C, Kornberg TB: **Proteolysis that is inhibited by hedgehog targest cubitus interuptus protein to the nucleus and converts it to a repressor**. *Cell* 1997, **89**:1043-1053.

43. Ruiz i Altaba A: **Gli proteins encode context-dependent positive and negative functions: implications for development and disease**. *Development* 1999, **126**:3205-3216.

44. Sasaki H, Nishizaki Y, Hui C, Nakafuku M, Kondoh H: **Regulation of Gli2 and Gli3 activities by an amino-terminal repression domain: implication of Gli2 and Gli3 as primary mediators of Shh signaling**. *Development* 1999, **126**:3915-3924.

45. Dai P, Akimaru H, Tanaka Y, Maekawa T, Nakafuku M, Ishii S: **Sonic Hedgehog-induced activation of the Gli1 promoter is mediated by GLI3**. *J Biol Chem* 1999, **274**:8143-8152.

46. Wang B, Fallon JF, Beachy PA: **Hedgehog-regulated processing of Gli3 produces an anterior/posterior repressor gradient in the developing vertebrate limb**. *Cell* 2000, **100**:423-434.

47. Hynes M, Stone DM, Dowd M, Pitts-Meek S, Goddard A, Gurney A, Rosenthal A: **Control of cell pattern in the neural tube by the zinc finger transcription factor and oncogene Gli-1**. *Neuron* 1997, **19**:15-26.

48. Yoon JW, Liu CZ, Yang JT, Swart R, Iannaccone P, Walterhouse D: **GLI activates transcription through a herpes simplex viral protein 16-like activation domain**. *J Biol Chem* 1998, **273**:3496-3501.

49. Aza-Blanc P, Lin HY, Ruiz i Altaba A, Kornberg TB: **Expression of the vertebrate Gli proteins in Drosophila reveals a distribution of activator and repressor activities**. *Development* 2000, **127**:4293-4301.

50. Ruiz i Altaba A: **Combinatorial Gli gene function in floor plate and neuronal inductions by Sonic hedgehog**. *Development* 1998, **125**:2203-2212.

51. Karlstrom RO, Tyurina OV, Kawakami A, Nishioka N, Talbot WS, Sasaki H, Schier AF: **Genetic analysis of zebrafish gli1 and gli2 reveals divergent requirements for gli genes in vertebrate development**. *Development* 2003, **130**:1549-1564.

52. Park HL, Bai C, Platt KA, Matise MP, Beeghly A, Hui CC, Nakashima M, Joyner AL: **Mouse *Gli1* mutants are viable but have defects in SHH signaling in combination with a *Gli2* mutation**. *Development* 2000, **127**:1593-1605.

53. Bai JCB, Joyner AL: **Gli1 can rescue the *in vivo* function of Gli2**. *Development* 2001, **128**:5161-5172.

54. Hui CC, Slusarski D, Platt KA, Holmgren R, Joyner AL: **Expression of three mouse homologs of the Drosophila segment polarity gene cubitus interruptus, Gli, Gli-2, and Gli-3, in ectoderm- and mesoderm-derived tissues suggests multiple roles during postimplantation development**. *Dev Biol* 1994, **162**:402-413.

55. Mo R, Freer AM, Zinyk DL, Crackower MA, Michaud J, Heng HH, Chik KW, Shi XM, Tsui LC, Cheng SH *et al.*: **Specific and redundant functions of Gli2 and Gli3 zinc finger genes in skeletal patterning and development**. *Development* 1997, **124**:113-123.

56. Matise MP, Epstein DJ, Park HL, Platt KA, Joyner AL: **Gli2 is required for induction of floor plate and adjacent cells, but not most ventral neurons in the mouse central nervous system**. *Development* 1998, **125**:2759-2770.

57. Ding Q, Motoyama J, Gasca S, Mo R, Sasaki H, Rossant J, Hui CC: **Diminished Sonic hedgehog signaling and lack of floor plate differentiation in Gli2 mutant mice**. *Development* 1998, **125**:2533-2543.

58. • Motoyama J, Milenkovic L, Iwama M, Shikata Y, Scott MP, Hui CC: **Differential requirement for Gli2 and Gli3 in ventral neural cell fate specification**. *Dev Biol* 2003, **259**:150-161.
Evidence that Gli2 and Gli3 behave differently along the anterior–posterior axis of the neural tube being partly redundant.

59. • Bai CB, Auerbach W, Lee JS, Stephen D, Joyner AL: ***Gli2*, but not *Gli1* is required for initial Shh signaling and ectopic activation of the Shh pathway**. *Development* 2002, **129**:4753-4761.
Evidence that Gli2 has an initial function in mediating Shh signaling. See also [56,57].

60. Litingtung Y, Chiang C: **Specification of ventral neuron types is mediated by an antagonistic interaction between Shh and Gli3**. *Nat Neurosci* 2000, **3**:979-985.

61. Gunther T, Struwe M, Aguzzi A, Schughart K: **Open brain, a new mouse mutant with severe neural tube defects, shows altered gene expression patterns in the developing spinal cord**. *Development* 1994, **120**:3119-3130.

62. Eggenschwiler JT, Anderson KV: **Dorsal and lateral fates in the mouse neural tube require the cell-autonomous activity of the open brain gene**. *Dev Biol* 2000, **227**:648-660.

63. Eggenschwiler JT, Espinoza E, Anderson KV: **Rab23 is an essential negative regulator of the mouse Sonic hedgehog signalling pathway**. *Nature* 2001, **412**:194-198.

64. Bose J, Grotewold L, Ruther U: **Pallister-Hall syndrome phenotype in mice mutant for Gli3**. *Hum Mol Genet* 2002, **11**:1129-1135.

65. Meyer NP, Roelink H: **The amino-terminal region of Gli3 antagonizes the Shh response and acts in dorsoventral fate specification in the developing spinal cord**. *Dev Biol* 2003, **257**:343-355.

66. Placzek M, Jessell TM, Dodd J: **Induction of floor plate differentiation by contact-dependent, homeogenetic signals**. *Development* 1993, **117**:205-218.

67. Charrier JB, Lapointe F, Le Douarin NM, Teillet MA: **Anti-apoptotic role of Sonic hedgehog protein at the early stages of nervous system organogenesis**. *Development* 2001, **128**:4011-4020.

68. Briscoe J, Pierani A, Jessell TM, Ericson J: **A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube**. *Cell* 2000, **101**:435-445.

69. Rowitch DH, S-Jacques B, Lee SM, Flax JD, Snyder EY, McMahon AP: **Sonic hedgehog regulates proliferation and inhibits differentiation of CNS precursor cells**. *J Neurosci* 1999, **19**:8954-8965.

70. Rallu M, Machold R, Gaiano N, Corbin JG, McMahon AP, Fishell G: **Dorsoventral patterning is established in the telencephalon of mutants lacking both Gli3 and Hedgehog signaling**. *Development* 2002, **129**:4963-4974.

71. Rodriguez I, Basler K: **Control of compartmental affinity boundaries by hedgehog**. *Nature* 1997, **389**:614-618.

72. Yu WP, Collarini EJ, Pringle NP, Richardson WD: **Embryonic expression of myelin genes: evidence for a focal source of oligodendrocyte precursors in the ventricular zone of the neural tube**. *Neuron* 1994, **12**:1353-1362.

73. Borycki A, Brown AM, Emerson CP Jr: **Shh and Wnt signaling pathways converge to control Gli gene activation in avian somites**. *Development* 2000, **127**:2075-2087.

74. Methot N, Basler K: **Hedgehog controls limb development by regulating the activities of distinct transcriptional activator and repressor forms of Cubitus interruptus**. *Cell* 1999, **96**:819-831.

75. Barnes EA, Kong M, Ollendorff V, Donoghue DJ: **Patched1 interacts with cyclin B1 to regulate cell cycle progression**. *EMBO J* 2001, **20**:2214-2223.

76. McMahon JA, Takada S, Zimmerman LB, Fan CM, Harland RM, McMahon AP: **Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite**. *Genes Dev* 1998, **12**:1438-1452.

77. Liem KF Jr, Tremml G, Roelink H, Jessell TM: **Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm**. *Cell* 1995, **82**:969-979.

78. Ruiz i Altaba A, Jessell TM: **Retinoic acid modifies the pattern of cell differentiation in the central nervous system of neurula stage *Xenopus* embryos**. *Development* 1991, **112**:945-958.

79. Franco PG, Paganelli AR, Lopez SL, Carrasco AE: **Functional association of retinoic acid and hedgehog signaling in *Xenopus* primary neurogenesis**. *Development* 1999, **126**:4257-4265.

80. Pierani A, Brenner-Morton S, Chiang C, Jessell TM: **A sonic hedgehog-independent, retinoid-activated pathway of neurogenesis in the ventral spinal cord**. *Cell* 1999, **97**:903-915.

81. Franz T: **Extra-toes (Xt) homozygous mutant mice demonstrate a role for the Gli-3 gene in the development of the forebrain**. *Acta Anat* 1994, **150**:38-44.

82. Theil T, Alvarez-Bolado G, Walter A, Ruther U: **Gli3 is required for Emx gene expression during dorsal telencephalon development**. *Development* 1999, **126**:3561-3571.