seminars in **CELL & DEVELOPMENTAL BIOLOGY,** Vol 10, 1999: pp. 353–362
Article No. scdb.1999.0295, available online at http://www.idealibrary.com on IDEAL®

The specification of neuronal identity by graded sonic hedgehog signalling

James Briscoe and Johan Ericson†*

During the development of vertebrate nervous system, distinct classes of motor neurons and interneurons are generated at distinct dorsoventral positions in the ventral neural tube. The differentiation of these neuronal subtypes is directed by the secreted protein Sonic Hedgehog (Shh). Shh acts in a graded manner to establish different neural progenitor cell populations, defined by the expression of homeodomain transcription factors. These factors are critical for the interpretation of graded Shh signalling and act initially both to refine progenitor domain boundaries and to maintain their integrity. Subsequently, these factors direct the expression of genes that confer neuronal subtype identity to post-mitotic neurons.

Key words: hedgehog/morphogen/motor neuron/neural patterning/ventral neural tube

THE VERTEBRATE NERVOUS SYSTEM develops from a sheet of neuroepithelial cells, the neural plate, that initially form a group of multipotent proliferating progenitors. As development proceeds the neural plate folds to form the neural tube and concomitantly, a pattern of regional cell identities along the dorsal–ventral axis becomes apparent (Figure 1). Subsequently, distinct progenitor populations begin to generate post-mitotic neurons that acquire characteristic identities and the distinct functional properties that underlie the assembly of functional neural circuits (refs 1–3; Figure 1).

At caudal levels of the neural tube, cells in the dorsal half generate different neurons that relay sensory information whereas cells in the ventral half generate distinct classes of motor neurons and ventral interneurons that participate in the co-ordination of motor output. These different classes of motor neurons and interneurons arise at characteristic dorsal–ventral positions in the ventral neural tube together with a specialized group of cells, the floor plate, that occupy the ventral mid-line (Figure 1). The generation of these cell types is dependent on a signal provided initially by axial mesodermal cells of the notochord that underlie the neural tube and subsequently by cells of the floor plate (refs 4,5; Figure 2A). This signal is mediated by the secreted glycoprotein Sonic hedgehog (Shh), a homologue of the *Drosophila* segment polarity gene *Hedgehog* (Hh). Shh is produced by the notochord and floor plate at the times that these two cell groups exhibit their inductive capacity (refs 6–8; Figure 2A). Moreover, gain and loss of function experiments indicate that Shh is both necessary and sufficient to induce ventral cell types.[9–12]

In this review, we will summarize evidence suggesting that Shh acts as a gradient morphogen to pattern the ventral neural tube. Initially, graded Shh signalling acts to establish domains of progenitor cells delineated by the expression of homeodomain transcription factors. These factors act to establish and maintain progenitor cell domains and subsequently, to regulate the expression of neuronal genes that specify the identity of post-mitotic neurons.

Sonic hedgehog can act as a gradient morphogen

How might a single factor control the identity and pattern of cell types generated in the ventral neural tube? Hedgehog protein activity has been shown to pattern a number of different tissues in both vertebrates and invertebrates.[13,14] In some instances, such

From the Howard Hughes Medical Institute, Dept. of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032, USA and †Dept. of Cell and Molecular Biology, Karolinska Institute, S-17177, Stockholm, Sweden*

1084-9521/99/030353+10 $30.00/0

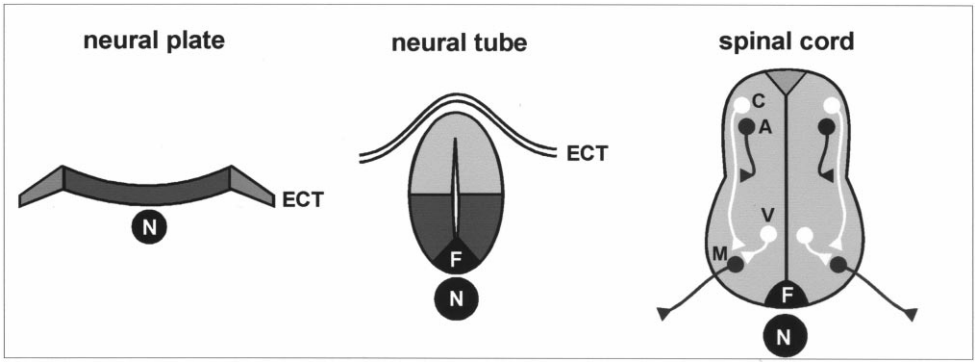

Figure 1. Stages of development of the spinal cord. The neural plate is generated as columnar epithelium flanked by ectoderm. The notochord (N) underlies the ventral midline of the neural plate. As development proceeds the neural plate folds to form a tube and the floor plate (F) differentiates at the ventral midline. Neuroepithelial cells proliferate and a pattern of regional identity becomes evident along the dorsal–ventral axis. Subsequently progenitors differentiate into functionally distinct neurons located at particular dorsal–ventral positions; subclasses include (C) Commissural neurons, (A) Association neurons, (M) Motor neurons and (V) Ventral interneurons.

as the *Drosophila* wing imaginal disc, a relay mechanism appears to operate; Hh acts locally to induce expression of a downstream secreted molecule, which then acts over a longer range to pattern the tissue[15] (Strigini and Cohen, this issue). In other cases, for example the *Drosophila* abdomen, Hh itself can act directly over a long range to impart positional information on responding cells.[16,17] In the ventral neural tube, several lines of evidence, discussed below, indicate that Shh acts in a graded manner, over a long range, to induce distinct cell types at different concentration thresholds (ref 18; Figure 2B).

Shh appears able to diffuse from the notochord and floor plate and to act directly on cells some distance away. First, signals from the notochord or floor plate induce motor neurons in transfilter assays.[19] This activity is blocked by an antibody against Shh, identifying this diffusible activity as Shh.[12] Furthermore, the expression of a number of Shh-responsive genes are regulated at a significant distance from the source of Shh.[18,20] Finally, the differentiation of motor neurons requires the continued presence of Shh well into S phase of the final cell division, suggesting a prolonged and direct requirement for Shh in responding cells.[12] Although the diffusion of Shh extracellularly seems to be limited by cholesterol and palmitoyl modifications,[21,22] the data suggest that low levels of Shh are able to diffuse and act over a range of several cell diameters. How a gradient of Shh signalling is established is currently not clear. Recently, however, a gene, *tout velu*, has been identified which appears to facilitate the diffusion of Hh in *Drosophila*,[23] possibly by regulating the interaction of Hh with extracellular matrix components.

The operation of a Shh activity gradient is suggested by the induction of ventral cell types in explants of naïve neural plate tissue exposed to recombinant Shh protein. In these studies, different cell types are generated in response to defined concentrations of Shh.[18] The cell types generated in response to a particular concentration of Shh correspond to the dorsal–ventral position at which these cells are generated *in vivo* (ref 18; Figure 2B). Thus, cell types that arise at a greater distance from the source of Shh *in vivo* are induced *in vitro* by lower concentrations of Shh than are cell types that differentiate closer to the source of Shh. Moreover, approximately twofold differences in the concentration of Shh are sufficient to elicit a switch between the cell types that are generated. Together, these results support a model in which Shh secreted by the notochord and floor plate diffuses into the adjacent neural epithelium and acts directly on responding cells, inducing different neuronal subtypes at different concentration thresholds.

Transduction of the Shh signal

How is the extracellular concentration of Shh perceived by responding cells? Mutagenesis screens in *Drosophila* have identified a number of genes notably *patched* (*ptc*), *smoothened* (*smo*), *cubitus interruptus* (*ci*)

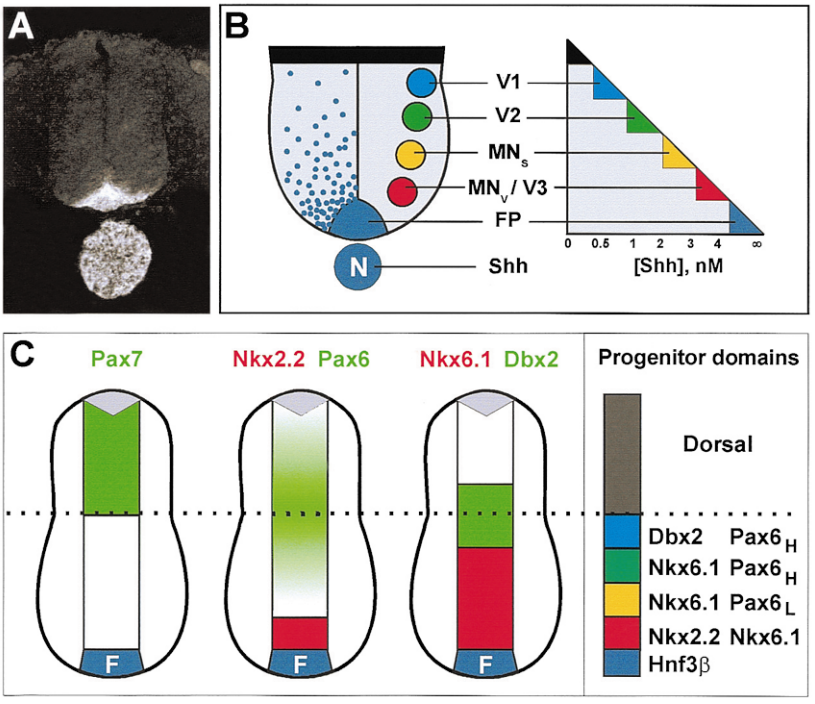

Figure 2. (*See legend overleaf*)

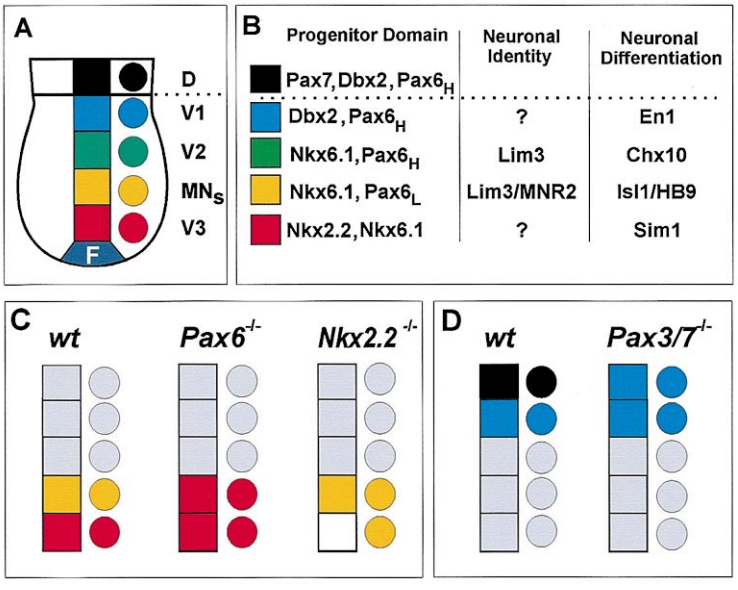

Figure 3. (*See legend overleaf*)

and *protein kinase A* (*pka*) that are involved in Hh signalling.[24] Ptc and Smo are transmembrane proteins that form a complex on the cell surface, with Ptc functioning to inhibit Smo activity.[25] Ptc functions as a Hh receptor subunit and ligand binding releases Smo from the effects of Ptc inhibition, permitting a signal to be transduced intracellularly.[25–27] Inside the cell, Hh signalling appears

to converge on the zinc finger transcription factor Ci.[24,28] In the presence of Hh, Ci is stabilized and activated and induces target gene expression.[24,28]

In vertebrates, homologues of Ptc, Smo, Ci and PKA have also been implicated in transducing Shh signals.[24,29] Of particular relevance, three vertebrate proteins, Gli, Gli-2 and Gli-3, which have homology to Ci, have been identified. Mice with a targeted deletion of Gli-2 fail to develop a floor plate.[30,31] Moreover, binding sites for Gli proteins have been identified in the enhancer of a gene required for floor plate specification, HNF3β and these are sufficient to direct expression of a reporter to the floor plate.[32] These data provide evidence for a role of Gli proteins in transducing a Shh signal. However, there is no data yet to indicate whether Shh directly regulates Gli protein activity.

The simplest model to account for the graded activity of Shh would be the activation of different levels of Gli protein activity in response to specific concentrations of Shh. Genes that respond to distinct levels of Shh signalling would contain enhancers responsive to different levels of Gli activity. In *Drosophila* embryos, such a model appears to account, at least in part, for the expression of genes in response to the gradient morphogen Bicoid.[33]

Nevertheless, although the floor plate is absent in mice containing mutations in both Gli and Gli-2, most ventral neuronal types including somatic motor neurons and interneurons are generated.[31] It is unlikely that Gli-3 substitutes for Gli and Gli-2, as Gli-3 is not expressed in the ventral neural tube.[31] Moreover, Gli-3 has been postulated to act as a repressor of Shh signalling.[29] It remains possible that there are additional Gli proteins in vertebrates. Alternatively, Gli proteins might not mediate all responses to Shh, in which case additional signalling pathways may be involved in Shh signal transduction. Consistent with this, it appears that transcription factors other than Ci are involved in transducing Hh signals in *Drosophila*.[34] In vertebrates, Krishnan *et al.* have suggested that Shh signalling activates a novel transcription factor via a protein phosphatase.[35] Additionally, cholesterol analogues, such as cyclopamine and jervine inhibit Shh signal transduction, implicating a role for cholesterol in Shh transduction,[36,37] this involvement could be in the activation of Gli proteins or other transcription factors. Together, these data raise the possibility that Shh signalling activates transcription factors other than, or in addition to Gli proteins, and that the graded response to Shh is mediated by a combination of intracellular signal transduction pathways.

Interpreting graded Shh signalling

How does graded Shh signalling control the identity and pattern of cell types generated in the ventral neural tube? In the following sections, we discuss evidence that suggests a model that outlines how graded Shh signalling might initiate the process that establishes the eventual pattern and identity of neurons in the ventral neural tube. The model can be divided into three steps (Figure 4). First, graded Shh signalling initiates neuronal patterning by establish-

Figure 2. Generation of neuronal pattern and graded Shh signaling. (A) Shh is expressed by cells of the notochord (N) and floor plate (F) and is responsible for specifying neuronal subtype identity and pattern in the ventral neural tube. (B) Shh acts as a gradient morphogen inducing different types of ventral neurons at different concentrations. V1 interneurons, V2 interneurons, sMNs and V3 interneurons (in the spinal cord) or visceral MNs (in the hindbrain) are generated at distinct dorsal-ventral positions in the spinal cord. The concentration of Shh that induces each type of neuron *in vitro* corresponds to the position at which it is generated *in vivo* — schematic based on the French Flag Model of L. Wolpert. (C) Progenitor domains of the ventral spinal cord. HNF3β is expressed by the floor plate, Pax7 expression defines the dorsal half of the neural tube. Pax6 and Nkx2.2 expression define abutting progenitor domains in the ventral neural tube. Distinct abutting domains of Nkx6.1 and Dbx2 also define progenitors in the neural tube. The combinatorial expression of homeodomain proteins divides progenitors in the ventral neural tube into distinct progenitor cell domains. These domains give rise to distinct neuronal progeny.

Figure 3. Generation of neurons from progenitor domains. (A) Distinct types of neurons are generated from each of the progenitor domains. (B) Summary of homeodomain proteins expressed by progenitor domains, neuronal identity genes and neuronal differentiation genes expressed by different neuronal subtypes. (C) Change in ventral cell fate in the neural tube of *Pax6* and *Nkx2.2* null mice. In wild type mice V3 interneurons are generated from Nkx2.2$^+$ progenitors and sMNs from Pax6$^+$ progenitors. In Pax6 null mice the domain of Nkx2.2 expression expands dorsally resulting in the dorsal expansion of V3 interneurons at the expense of sMNs. In Nkx2.2 null mice V3 interneurons are lost and there is a ventral expansion in the generation of sMNs. (D) *Pax7/Pax3* and the generation of V1 interneurons. In wild-type mice V1 interneurons are generated from Dbx2$^+$ progenitors ventral to the *Pax 3/7* expressing domain. In mice lacking both *Pax3* and *Pax7* the domain of V1 generation expands dorsally.

ing the restricted expression of a number of homeodomain proteins that define distinct dorsal–ventral domains of progenitor cells. Second, interactions between homeodomain proteins in neighboring domains act to refine and maintain the integrity of progenitor cell domains. Finally, as cells leave the cell cycle and differentiate, homeodomain proteins expressed in progenitor cells direct the activation of neuronal identity determining genes to the appropriate dorsal–ventral regions of the neural tube.

Progenitor cell domains

At the time that neuronal differentiation is initiated, the ventral neural tube can be divided into at least four neuronal progenitor cell domains that each gives rise to distinct classes of neurons (Figure 2B,C). These progenitor cell domains can be defined, either alone or in combination, by the expression a number of homeodomain proteins. The expression of these transcription factors is regulated by graded Shh signalling; we have divided these genes into two groups based on their regulation by Shh. First, there is a set of genes that are repressed by Shh, we term these Class I genes. Genes within this class include homeodomain proteins of the *Pax*-, *Msx*- and *Dbx*-families (Figure 4). The second group, termed Class II genes, are dependent on Shh signalling for their expression and include members of the *Nkx*-family of transcription factors. Although the expression of these two classes of genes is dependent on Shh signalling it remains possibly that the transcriptional control of some of the genes within these groups is indirect (see below).

Establishing progenitor cell domains: regulation of class I genes

Initially, most or all cells within the caudal neural plate express the homeodomain proteins Pax3, Pax7, Msx1 and Msx2. The expression of these genes is then rapidly repressed, restricting their expression to

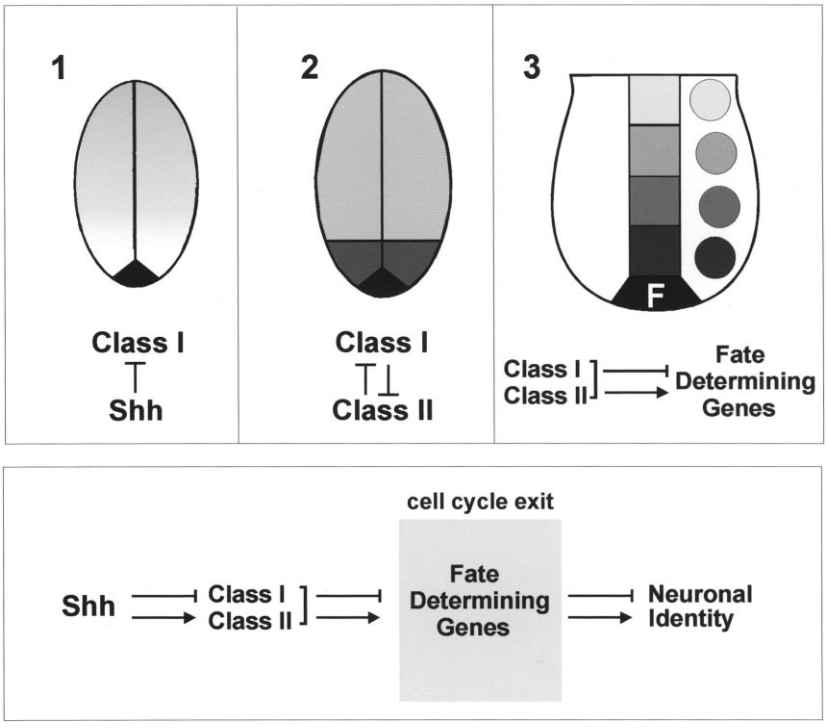

Figure 4. A model for patterning the ventral neural tube. (1) Initially, graded Shh signaling acts to repress the expression of a number of genes (Class I), this allows the induction of Shh responsive genes (Class II). (2) Homeodomain proteins expressed by adjacent progenitor domains form repressive feedback loops that act to refine and maintain progenitor cell domains. (3) Subsequently, genes expressed by progenitor cells regulate the activation of cell fate determining genes that confer neuronal identity to post-mitotic neurons.

the dorsal half of the newly formed neural tube.[12,38] This repression is mediated by low levels of Shh signalling, resulting in the generation of a group of ventral progenitor cells that maintain the capacity to give rise to all ventral cell types (ref 12; Figure 2C). Similarly, Pax6 is initially expressed in the entire neural plate with the exception of the presumptive floor plate. Expression is then repressed from the most ventral regions to leave a dorsal (high)–ventral (low) gradient of expression (ref 39; Figure 2C). Complete repression of Pax6, however, requires high levels of Shh signalling and the domain of Pax6 expression therefore extends more ventrally than Pax7. In addition, Dbx2 is repressed by a concentration of Shh intermediate between the concentrations required to repress Pax6 and Pax7 (ref 40; Figure 2C). Therefore, an initial step in the regionalization of the ventral neural tube appears to involve the differential sensitivity of these Class I genes to repression by Shh (Figure 4).

Establishing progenitor cell domains: regulation of class II genes

The induction of Class II genes, comprising members of the *Nkx*-family, is dependent on Shh signalling. However, this dependence appears to be, at least in part, indirect and requires the repression of Class I genes. For example, Nkx2.2 is expressed by progenitors adjacent to the floor plate and abutting the ventral limit of Pax6 expression (refs 39,41; Figure 2C). Thus, expression of Pax6 and Nkx2.2 define two mutually exclusive progenitor cell domains and Shh signalling is necessary and sufficient to establish these two domains.[39] In *Pax6* deficient mice (*Small eye* mutants), the domain of expression of Nkx2.2 expands dorsally (Figure 3C) although the level of Shh signalling is unchanged. This indicates that the expression of Nkx2.2 is regulated indirectly by the Shh mediated repression of Pax6.[39]

The expression of Nkx6.1 and Dbx2 display a similar mutually exclusive relationship (Figure 2C). Nkx6.1 is induced by Shh signalling[42] and the expression domain appears to extend up to the ventral boundary of Dbx2 expression (refs 40,42; Figure 2C). Therefore, unlike Nkx2.2, Nkx6.1 is not sensitive to repression by Pax6. Rather it is possible that Dbx2, or another gene expressed in this domain, controls the dorsal limit of Nkx6.1 expression. These observations raise the possibility that the expression domain of Shh-dependent genes (Class II genes) is largely controlled by transcription factors that are repressed (Class I genes) at different concentration thresholds of Shh.

Whether the expression of Shh-induced genes, such as *Nkx2.2* or *Nkx6.1* requires direct Shh signalling is not clear. The expression of Nkx2.2 in *Pax6* mutant mice is not de-repressed in all cells that normally express Pax6.[39] The dorsal limit of Nkx2.2 expression, therefore, may reflect the position at which another Nkx2.2 repressing gene is expressed or the position at which Shh activity falls below a threshold necessary to activate *Nkx2.2* transcription. In the simplest case, the expression of Nkx2.2 and Nkx6.1 genes could be de-repressed following the Shh mediated repression of Pax6 and Dbx2, respectively. De-repression mechanisms have been implicated in other tissues patterned by morphogen gradients. For example, during anterior–posterior patterning of the *Drosophila* embryo the expression of certain Gap genes is controlled by ubiquitous activators while spatial control is achieved by repression mediated by other Gap genes.[43] Similarly, the activation of presumed dpp target genes in anterior–posterior patterning of *Drosophila* wings appears to be indirect and achieved through the Dpp mediated repression of the putative transcriptional repressor, *brinker*.[44]

Refining and maintaining progenitor cell domains

Following the initial Shh mediated establishment of distinct progenitor cell domains, interactions between Class I and Class II genes expressed by the different domains seem to act to refine and maintain the integrity of these domains (Figure 3). Recent data suggest that the mutually exclusive relationship between Pax6 and Nkx2.2 is maintained by reciprocal repressive interactions between the two genes. The ectopic expression of Nkx2.2 in the chick neural tube results in the repression of Pax6. In contrast, Pax7, which is expressed in a domain that never abuts the Nkx2.2 domain, is not affected by Nkx2.2 expression.[45] These data suggest that a specific mutually repressive feedback loop is established between Pax6 and Nkx2.2. Pax6 expression does not expand ventrally in *Nkx.2.2* mutant mice.[50] However, *Nkx2.9*, a gene related to *Nkx2.2*, is expressed transiently in the same domain raising the possibility of redundancy between these two genes.[50]

The strikingly sharp boundaries of gene expression delimiting other ventral progenitor domains raise the possibility that similar repressive feedback loops might operate between genes expressed in these abutting progenitor domains. Such a mechanism could be

important for refining boundaries between progenitor cell domains and could contribute to the production of discrete patterns of gene expression from an initially crude gradient of extracellular ligand. Furthermore, during development, there is considerable growth of the ventral neuroepithelium and it is difficult to envision how graded Shh signalling, alone, could account for the maintenance of these domains. Repressive feedback loops, therefore, could function to maintain the integrity of progenitor cell domains throughout the development of the CNS. Such a mechanism would remove the requirement for prolonged graded Shh signalling. This does not exclude, however, a role for Shh at late stages of neural patterning. It is possible that a mitogenic activity of Shh[46] at these later stages acts to expand the earlier established progenitor cell domains.

The principles of this model are reminiscent of the establishment of anterior–posterior polarity within the *Drosophila* embryo.[43,47] The graded distribution of two transcription factors, Bicoid and Caudal, initiates the regionally restricted expression of Gap genes. The syncytial organization of the early *Drosophila* embryo removes the requirement for a secreted extracellular morphogen. Cross-regulation between Gap genes, then, acts to establish and maintain sharp boundaries of gene expression resulting in defined domains of Gap gene expression. The similarity of regulation and function of Gap genes and progenitor cell domain genes raises the possibility that this mechanism represents a general strategy used by tissues in response to morphogen gradients to generate discrete domains of gene expression.

2.5. Specification of neuronal subtype identity

Different ventral progenitor cell domains generate neurons with distinct identities (Figure 3A,B). Most dorsally in the ventral neural tube, V1 interneurons are derived from progenitor cells ventral to the Pax7 boundary that express Pax6 and Dbx2.[39,48] V2 interneurons are generated from progenitor cells expressing Nkx6.1 and high Pax6 levels,[39,49] whereas somatic motor neurons (sMN) are derived from progenitors that express Nkx6.1 and low levels of Pax6.[39,49] The ventral most neuronal progenitor domain, defined by Nkx2.2 and Nkx6.1 expression, generates V3 interneurons in the spinal cord and visceral motor neurons (vMNs) in the anterior cervical spinal cord and hindbrain.[50] Transcription factors that define distinct progenitor cell populations are generally down-regulated in cells as they leave the cell cycle to differentiate into neurons.[51] This observation raises the question of how genes expressed in progenitor cell domains impart subtype identity to differentiating neurons.

Studies of sMN induction have indicated that sMN progenitor cells depend upon the ambient Shh concentration up to the final cell division.[12] This transition from a Shh-dependent to a Shh-independent state correlates with the induction of the homeodomain protein MNR2 during the final cell cycle of sMN progenitors.[49] MNR2 is sufficient to induce MN differentiation independent of position and developmental history of cells when ectopically expressed in the chick spinal cord.[49] The expression of two closely related LIM-homeodomain proteins, Lim3 (Lhx3) and Gsh4 (Lhx4) are also initiated during the final cell division of sMNs and V2 interneurons.[52] Ectopic expression of Lim3 induces V2 interneurons in chick spinal cord but is not sufficient to induce MNs.[49] However, Lim3 and Gsh4 are involved in sMN differentiation: Lim3 together with Is11, an additional transcription factor expressed by sMNs, can induce the expression of certain genes characteristic of sMNs.[49] Moreover, in mice lacking Lim3 and Gsh4 function, sMNs appear to acquire an identity characteristic of visceral MNs (spinal accessory MNs) that are normally generated from Nkx2.2 progenitor cells rather than Pax6 progenitors.[39,52] Together, these results suggest that transcription factors, such as MNR2 and Lim3 comprise a group of neuronal identity factors that determine the identity of post-mitotic neurons (Figure 3B, Figure 4).

Since neuronal identity genes can specify cell fate independent of dorsal–ventral position and Shh concentration, a major role for the patterning genes expressed in progenitor cells must be to direct the activation of neuronal identity genes to the appropriate dorsal–ventral region of the neural tube. Recent data supports this idea. In *Pax6* mutant mice, there is a dorsal-to-ventral switch in neuronal subtype identity that results in a dorsal expansion and increased numbers of V3 interneurons in the spinal cord and vMNs in the hindbrain (refs 39,53; Figure 3C). At both levels of the neuraxis, this increase occurs at the expense of sMNs. Several lines of evidence suggest that expansion of Nkx2.2 expression is responsible for the switch in cell fate in *Pax6* mutant mice. First, at caudal spinal cord levels of *Pax6* mutant mice where the expansion of Nkx2.2 is not as pronounced, sMNs are generated albeit in reduced numbers.[39,53] Second, in the spinal cord of mice lacking Nkx2.2, although the expression of Pax6 is unchanged, there

is a reciprocal ventral-to-dorsal cell fate switch resulting in the generation of sMNs adjacent to the floor plate at the expense of V3 interneurons.[50] Thus, Pax6 is not directly required for the generation of sMNs. Finally, the ectopic expression of Nkx2.2 in chick spinal cord indicates that Nkx2.2 is sufficient to repress sMN generation and induce the expression of *Sim1*, a marker of V3 interneurons.[45] Therefore, within the ventral spinal cord, Nkx2.2 appears to be critical for the interpretation of the Shh gradient and governs the choice between V3 interneuron and sMN generation. The loss of *Nkx2.2* results in the generation of neuronal progeny characteristic of exposure to lower levels of Shh signalling whereas the ectopic expression of Nkx2.2 results in the generation of V3 interneurons independent of Shh signalling. Nkx2.2 appears therefore to control the expression of neuronal identity genes, such as MNR2 and Lim3. Indeed, Nkx2.2 is sufficient to repress MNR2 and Lim3 in the chick spinal cord[45] and Lim3 expands ventrally in mice lacking Nkx2.2 activity.[50]

Since Pax6 is not directly required for sMN differentiation, the data raise the question of which genes activate MNR2 and Lim3 to promote sMN generation. The expression domain of Nkx6.1 coincides with the positions at which V2 interneurons and sMNs are generated (Figure 2C, Figure 3). It is possible, therefore, that Nkx6.1 is involved in the induction of one or both of these neuronal subtypes. If this is the case, an additional gene(s) is required to distinguish sMNs and V2 interneurons to account for the selective activation of MNR2 in sMNs. Genes that control the fate of other neuronal subtypes remain largely unknown. However, Pax6, Pax3 and Pax7 appear to be involved in the control of V1 interneuron differentiation. V1 interneurons are generated from $Pax6^+/Dbx2^+$ progenitors ventral to the Pax7/Pax3 boundary but not from $Pax6^+/Dbx2^+$ progenitors that reside within the Pax3/Pax7 domain.[39,40,54] In Pax6 null mice, V1 interneurons are missing indicating a requirement for Pax6 in generating these neurons.[39,54] In contrast, in mice lacking both Pax3 and Pax7, the domain of V1 interneuron generation expands dorsally and appears to occupy the entire Dbx2 expression domain (ref 55; Figure 3D). Therefore, it is likely that Pax6 and Dbx2 act to induce neuronal identity genes necessary for V1 generation whereas Pax3 and Pax7 repress this activity,

Together the data discussed above, although fragmentary, begin to reveal a genetic network that controls neuronal cell fate decisions in the ventral neural tube. The control of neuronal subtype identity appears to be regulated by a relatively small number of neuronal identity genes. The expression of these genes is directed to the appropriate dorsal–ventral regions by the combined positive and negative activities of transcription factors expressed in progenitor cells (Figure 4). Since neuronal identity genes are activated only during the final cell division of neurons, their activation must be coordinated with a general neurogenic program.[2] In conclusion, in addition to defining progenitor cell domains, the genes expressed by progenitor cells appear to regulate neuronal identity genes that subsequently specify the identity of MNs and interneurons generated in the ventral neural tube.

Neuronal diversity in the ventral neural tube

In this review we have focused on how graded Shh signalling might act to establish the pattern of neuronal subtypes generated in the ventral neural tube. The data begin to provide an explanation for how small differences in the concentration of an extracellular ligand can be translated into distinct patterns of gene expression. The observations suggest a relatively simple and possibly general principle for neuronal patterning (Figure 4). Graded Shh initiates the regionally restricted expression of patterning genes in mitotic progenitors. Subsequently, these patterning genes act to refine and maintain the integrity of progenitor cell domains during development. During the period of neurogenesis, the activities of patterning genes function to direct the expression of neuronal identity genes which then function to specify neuronal identity and confer a program of differentiation resulting in the acquisition of distinct functional properties by post-mitotic neurons.

In addition to the role of Shh, it appears that several other mechanisms operate to influence cell fate decisions in the ventral neural tube. Studies of sMN and oligodendrocyte differentiation indicate that these cell types are generated from the same progenitor domain, but at different stages of neural tube development.[56] Similar to models proposed for cortical development, it is likely that this temporal switch in cell type identity reflects changes in the local microenvironment, over time. Moreover, it is possible that distinct subtypes of neurons are generated simultaneously from the same progenitor domain, suggesting that additional mechanisms act within individual progenitor domains to generate neuronal diversity.[54] In addition, the identity of ventral neurons is influ-

enced by signals that impart positional character along the anterior–posterior axis. This enables the corresponding dorsal–ventral domain at different anterior–posterior positions to generate neurons with distinct functional properties. Such a form of positional organization is particularly apparent in the control of MN subtype identity in the spinal cord and hindbrain and appears to be controlled by signals from the paraxial mesoderm regulating *Hox* gene expression.[57,58] Furthermore, neuronal fate can also be influenced after the cells have left the cell cycle, for example, by local environmental signals and signals from neuronal targets.[59,60] Therefore, although graded Shh signalling plays a pivotal role in ventral neuronal patterning, it appears that additional mechanisms operate to achieve the neuronal diversity necessary for the organization and function of the mature nervous system.

Acknowledgements

We thank A. Pierani for communicating results prior to publication and T. Jessell, B. Novitch and C. William for comments on the manuscript. J.B. is an Associate of the Howard Hughes Medical Institute. J.E. is supported by grants from the Swedish Foundation for Strategic Research and the Swedish Natural Science Research Council.

References

1. Placzek M, Furley A (1996) Patterning cascades in the neural tube. Neural development. Curr Biol 6:526–529
2. Tanabe Y, Jessell TM (1996) Diversity and pattern in the developing spinal cord. Science 274:1115–1123
3. Lee K, Jessell TM (1999) The specification of dorsal cell fates in the vertebrate central nervous system. Ann Rev Neurosci 22:261–294
4. Yamada T, Placzek M, Tanaka H, Dodd J, Jessell TM (1991) Control of cell pattern in the developing nervous system: polarizing activity of the floor plate and notochord. Cell 64:635–647
5. van Straaten HW, Thors F, Wiertz-Hoessels L, Hekking J, Drukker J (1985) Effect of a notochordal implant on the early morphogenesis of the neural tube and neuroblasts: histometrical and histological results. Dev Biol 110:247–254
6. Echelard Y, Epstein DJ, St-Jacques B, Shen L, Mohler J, McMahon JA, McMahon AP (1993) Sonic hedgehog, a member of a family of putative signalling molecules, is implicated in the regulation of CNS polarity. Cell 75:1417–1430
7. Krauss S, Concordet JP, Ingham PW (1993) A functionally conserved homolog of the *Drosophila* segment polarity gene hh is expressed in tissues with polarizing activity in zebrafish embryos. Cell 75:1431–1444
8. Roelink H, Augsburger A, Heemskerk J, Korzh V, Norlin S, Ruiz i Altaba A, Tanabe Y, Placzek M, Edlund T, Jessell TM, Dodd J (1994) Floor plate and motor neuron induction by vhh-1, a vertebrate homolog of hedgehog expressed by the notochord. Cell 76:761–775
9. Marti E, Bumcrot DA, Takada R, McMahon AP (1995) Requirement of 19K form of Sonic hedgehog for induction of distinct ventral cell types in CNS explants. Nature 375:322–323
10. Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, Westphal H, Beachy PA (1996) Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature 383:407–413
11. Roelink H, Porter JA, Chiang C, Tanabe Y, Chang DT, Beachy PA, Jessell TM (1995) Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of sonic hedgehog autoproteolysis. Cell 81:445–455
12. Ericson J, Morton S, Kawakami A, Roelink H, Jessell TM (1996) Two critical periods of Sonic Hedgehog signalling required for the specification of motor neuron identity. Cell 87:661–673
13. Hammerschmidt M, Brook A, McMahon AP (1997) The world according to hedgehog. Trends Genet 13:14–21
14. Lawrence PA, Struhl G (1996) Morphogens, compartments, and pattern: lessons from *Drosophila*. Cell 85:951–953
15. Nellen D, Burke R, Struhl G, Basler K (1996) Direct and long-range action of a DPP morphogen gradient. Cell 85:357–368
16. Heemskerk J, DiNardo S (1994) *Drosophila* hedgehog acts as a morphogen in cellular patterning. Cell 76:449–460
17. Struhl G, Barbash DA, Lawrence PA (1997) Hedgehog acts by distinct gradient and signal relay mechanisms to organise cell type and cell polarity in the *Drosophila* abdomen. Development 124:2155–2165
18. Ericson J, Briscoe J, Rashbass P, van Heyningen V, Jessell TM (1997) Graded sonic hedgehog signaling and the specification of cell fate in the ventral neural tube. Cold Spring Harb Symp Quant Biol 62:451–457
19. Tanabe Y, Roelink H, Jessell TM (1995) Induction of motor neurons by Sonic hedgehog is independent of floor plate differentiation. Curr Biol 5:651–658
20. Marigo V, Tabin CJ (1996) Regulation of patched by sonic hedgehog in the developing neural tube. Proc Natl Acad Sci USA 93:9346–9351
21. Porter JA, Young KE, Beachy PA (1996) Cholesterol modification of hedgehog signalling proteins in animal development. Science 274:255–259
22. Pepinsky RB, Zeng C, Wen D, Rayhorn P, Baker DP, Williams KP, Bixler SA, Ambrose CM, Garber EA, Miatkowski K, Taylor FR, Wang EA, Galdes A (1998) Identification of a palmitic acid-modified form of human Sonic hedgehog. J Biol Chem 273:14037–14045
23. Bellaiche Y, The I, Perrimon N (1998) Tout-velu is a *Drosophila* homologue of the putative tumour suppressor EXT-1 and is needed Hh diffusion. Nature 394:85–88
24. Ingham PW (1998) Transducing Hedgehog: The story so far. EMBO J 17:3505–3511
25. Stone DM, Hynes M, Armanini M, Swanson TA, Gu Q, Johnson RL, Scott MP, Pennica D, Goddard A, Phillips H, Noll M, Hooper JE, de Sauvage F, Rosenthal A (1996) The tumour suppressor gene patched encodes a candidate receptor for Sonic hedgehog. Nature 384:129–134
26. Chen Y, Struhl G (1996) Dual roles for patched in sequestering and transducing Hedgehog. Cell 87:553–563
27. Marigo V, Davey RA, Zuo Y, Cunningham JM, Tabin CJ (1996) Biochemical evidence that patched is the Hedgehog receptor. Nature 384:176–179
28. Aza-Blanc P, Ramirezweber F, Laget M, Schwartz C, Kornberg T (1997) Proteolysis that is inhibited by hedgehog targets cubitus-interruptus protein to the nucleus and converts it to a repressor. Cell 89:1043–1053
29. Ruiz i Altaba A (1997) Catching a glimpse of hedgehog. Cell 90:193–196

30. Ding Q, Motoyama J, Gasca S, Mo R, Sasaki H, Rossant J, Hui CC (1998) Diminished Sonic hedgehog signalling and lack of floor plate differentiation in Gli2 mutant mice. Development 125:2533–2543
31. Matise MP, Epstein DJ, Park HL, Platt KA, Joyner AL (1998) Gli2 is required for induction of floor plate and adjacent cells, but not most ventral neurons in the mouse central nervous system. Development 125:2759–2770
32. Sasaki H, Hui C, Nakafuku M, Kondoh H (1997) A binding site for Gli proteins is essential for HNF-3β floor plate enhancer activity in transgenics and can respond to Shh *in vitro*. Development 124:1313–1322
33. Burz DS, Rivera-Pomar R, Jackle H, Hanes SD (1998) Cooperative DNA-binding by Bicoid provides a mechanism for threshold-dependent gene activation in the *Drosophila* embryo. EMBO J 17:5998–6009
34. Lessing D, Nusse R (1998) Expression of wingless in the *Drosophila* embryo: a conserved cis-acting element lacking conserved Ci-binding sites is required for patched-mediated repression. Development 125:1469–1476
35. Krishnan V, Pereira FA, Qiu Y, Chen CH, Beachy PA, Tsai SY, Tsai MJ (1997) Mediation of Sonic hedgehog-induced expression of COUP-TFII by a protein phosphatase. Science 278:1947–1950
36. Incardona JP, Gaffield W, Kapur RP, Roelink H (1998) The teratogenic Veratrum alkaloid cyclopamine inhibits sonic hedgehog signal transduction. Development 125:3553–3562
37. Cooper MK, Porter JA, Young KE, Beachy PA (1998) Teratogen-mediated inhibition of target tissue response to Shh signalling. Science 280:1603–1607
38. Liem KF Jr, Tremml G, Roelink H, Jessell TM (1995) Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. Cell 82:969–979
39. Ericson J, Rashbass P, Schedl A, Brenner-Morton S, Kawakami A, van Heyningen V, Jessell TM, Briscoe J (1997) Pax6 Controls Progenitor Cell Identity and Neuronal Fate in Response to Graded Shh signalling. Cell:90:169–180
40. Pierani A, Brenner-Morton S, Chiang C, Jessell TM (1999) Parallel retinoid and sonic hedgehog signaling pathways specify Dbx^+ progenitors and the generation of V1 interneurons in the embryonic spinal cord. Cell, in press
41. Price M, Lazzaro D, Pohl T, Mattei MG, Ruther U, Olivo JC, Duboule D, Di Lauro R (1992) Regional expression of the homeobox gene Nkx-2.2 in the developing mammalian forebrain. Neuron 8:241–255
42. Qiu M, Shimamura K, Sussel L, Chen S, Rubenstein JLR (1998) Control of anteroposterior and dorsoventral domains of Nkx-6.1 gene expression relative to other Nkx genes during vertebrate CNS development. Mech Dev 72:77–88
43. Rivera-Pomar R, Jackle H (1996) From gradients to stripes in *Drosophila* embryogenesis: filling in the gaps. Trends Genet 12:478–483
44. Campbell G, Tomlinson A (1999) Transduction of the Dpp morphogen gradient in the *Drosophila* wing: regulation of Dpp targets by Brinker. Cell 96:553–562
45. Briscoe J, Jessell TM, Ericson J (1999) The role of Nkx genes in the interpretation of graded Shh signaling and the specification of neuronal subtype identity. In preparation
46. Jensen AM, Wallace VA (1997) Expression of Sonic hedgehog and its putative role as a precursor cell mitogen in the developing mouse retina. Development 124:363–371
47. Akam M (1987) The molecular basis for metameric pattern in the *Drosophila* embryo. Development 101:1–22
48. Matise MP, Joyner AL (1997) Expression patterns of developmental control genes in normal and Engrailed-1 mutant mouse spinal cord reveal early diversity in developing interneurons. J Neurosci 17:7805–7816
49. Tanabe Y, William CM, Jessell TM. (1998) Specification of motor neuron identity by the MNR2 homeodomain protein. Cell 95:67–80
50. Briscoe J, Sussel L, Serup P, Hartigan-O'Connor D, Jessell TM, Rubenstein JLR, Ericson J (1998) Homeobox gene Nkx2.2 and specification of neuronal identity by graded Sonic hedgehog signalling. Nature 398:622–627
51. Goulding M (1998) Specifying Motor neurons and their connections. Neuron 21:943–946
52. Sharma K, Sheng HZ, Lettieri K, Li H, Karavanov A, Potter S, Westphal H, Pfaff SL (1998) LIM homeodomain factors Lhx3 and Lhx4 assign subtype identities for motor neurons. Cell 95:817–828
53. Osumi N, Hirota A, Ohuchi H, Nakafuku M, Emura T, Kuratani S, Fujiwara M, Noji S, Eto K (1997) Pax-6 is involved in the specification of hindbrain motor neuron subtype. Development 124:2961–2972
54. Burrill JD, Moran L, Goulding MD, Saueressig H (1997) PAX2 is expressed in multiple spinal cord interneurons, including a population of EN1 + interneurons that require PAX6 for their development. Development 124:4493–4503
55. Mansouri A, Gruss P (1998) Pax3 and Pax7 are expressed in commissural neurons and restrict ventral neuronal identity in the spinal cord. Mech Dev 78:171–178
56. Richardson WD, Pringle NP, Yu WP, Hall AC (1997) Origins of spinal cord oligodendrocytes: possible developmental and evolutionary relationships with motor neurons. Dev Neurosci 19:58–68
57. Ensini M, Tsuchida TN, Belting HG, Jessell TM (1998) The control of rostrocaudal pattern in the developing spinal cord: specification of motor neuron subtype identity is initiated by signals from paraxial mesoderm. Development 125:969–982
58. Lumsden A, Krumlauf R (1996) Patterning the vertebrate neuraxis. Science 274:1109–1115
59. Sockanathan S, Jessell TM (1998) Motor neuron-derived retinoid signaling specifies the subtype identity of spinal motor neurons. Cell 94:503–514
60. Lin JH, Saito T, Anderson DJ, Lance-Jones C, Jessell TM, Arber S (1998) Functionally-related motor neuron pool and muscle sensory afferent subtypes defined by coordinate ETS gene expression. Cell 95:393–407