motor neurons by postsynaptic Ca^{2+}-dependent mechanisms. *J. Neurosci.* 18, 9673–9684
13 Grant, P. *et al.* (2001) Cyclin-dependent protein kinase 5 (Cdk5) and the regulation of neurofilament metabolism. *Eur. J. Biochem.* 268, 1534–1546
14 Nixon, R.A. (1998) Dynamic behavior and organization of cytoskeletal proteins in neurons: reconciling old and new findings. *BioEssays* 20, 798–807
15 Bajaj, N.P. *et al.* (1999) Cyclin dependent kinase-5 (CDK-5) phosphorylates neurofilament heavy (NF-H) chain to generate epitopes for antibodies that label neurofilament accumulations in amyotrophic lateral sclerosis (ALS) and is present in affected motor neurones in ALS. *Prog. Neuropsychopharmacol. Biol. Psychiatr.* 23, 833–850
16 Evans, D.B. *et al.* (2000) Tau phosphorylation at serine 396 and serine 404 by human recombinant tau protein kinase II inhibits tau's ability to promote microtubule assembly. *J. Biol. Chem.* 275, 24977–24983
17 Ishihara, T. *et al.* (1999) Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. *Neuron* 24, 751–762
18 Zhao, C. *et al.* (2001) Charcot–Marie-tooth disease type 2a caused by mutation in a microtubule motor kif1bbeta. *Cell* 105, 587–597
19 Kwon, Y.T. *et al.* (2000) Regulation of N-cadherin-mediated adhesion by the p35-Cdk5 kinase. *Curr. Biol.* 10, 363–72
20 Bibb, J.A. *et al.* (2001) Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. *Nature* 410, 376–380
21 Ohshima, T. *et al.* (1996) Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology and perinatal death. *Proc. Natl. Acad. Sci. U. S. A.* 93, 11173–11178

Holger Patzke
Li-Huei Tsai*
Dept of Pathology, Harvard Medical School and Howard Hughes Medical Institute, Boston, MA 02115, USA.
*e-mail: li-huei_tsai@hms.harvard.edu

Hedgehog patterns midbrain ARChitecture

Robert Machold and Gord Fishell

Recent work from Agarwala *et al.* has uncovered exquisite ventral patterning in the mesencephalon. Using electroporation in chicks, they show that ectopic expression of Sonic Hedgehog (Shh) in dorsal mesencephalon can recapitulate this patterning in its entirety. These results are discussed in the context of the purported role of Shh as a morphogen.

A fundamental issue in the development of the central nervous system (CNS) is how different neuronal populations are specified along the dorsal–ventral axis of the neural tube. Although a number of signaling molecules have been implicated in this process, none have been as well studied as the secreted protein Sonic Hedgehog (Shh) [1]. Genetic ablation studies in mice have shown that Shh is essential for the formation of ventral-cell types throughout the rostral–caudal extent of the CNS [2,3]. During the development of the spinal cord, Shh is expressed in the notochord and floor-plate (FP) cells, and is required for the formation of the FP, motor neurons (MNs), and interneurons V0–V3 [4,5]. Studies performed on chick spinal-cord explants *in vitro* have demonstrated that these six ventral-cell types can be induced differentially by progressive two- to three-fold changes in the concentration of Shh, in a manner consistent with their position along the dorsal–ventral axis (Fig. 1) [1,6].

Despite this strong evidence that Shh can act as a morphogen *in vitro*, corresponding *in vivo* data supporting this hypothesis have been less compelling.

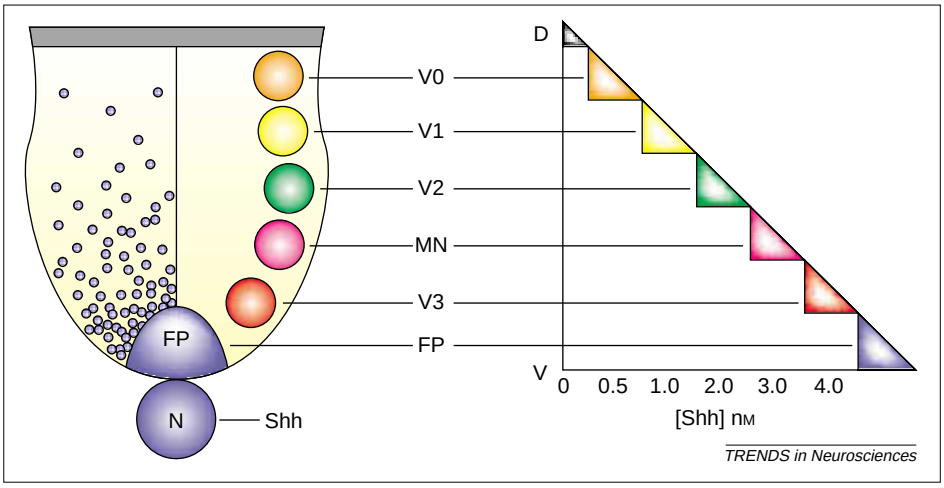

Fig. 1. This schematic shows the concentration-dependent induction of ventral spinal-cord cell types in response to Sonic Hedgehog (Shh). Although *in vitro* work in spinal cord has shown a clear relationship between the concentration of Shh and the types of cell induced, a corresponding *in vivo* demonstration that point-sources of Shh can organize surrounding tissues has been more difficult to obtain. Reproduced, with permission, from [1]. Abbreviations: N, notochord; FP, floor plate; V, ventral; MN, motor neuron; D, dorsal.

Ectopic expression studies of Shh in the spinal cord have been problematic with regard to demonstrating long-range behavior. Although the transgenic expression of Shh under the control of the Wnt-1 enhancer in mice induced expression of some ventral markers, it did not result in a dorsal recapitulation of graded ventral patterning [7]. A probable interpretation of this result is that Shh requires other molecules *in vivo* to execute its organizing function. Earlier studies on chick spinal-cord development found that grafts of either notochord (NC) or FP in dorsal locations could induce a graded ventral pattern, including MNs, ectopically [8]. In this regard, it is noteworthy that dorsal transplantation of the NC yielded an ectopic ventral pattern only in embryos in which the roof plate failed to form. This suggests that the dorsal midline is a source of molecules that antagonize Shh signaling. Probable candidates for such antagonists are the bone morphogenic proteins (BMPs), which have been implicated in patterning of the dorsal neural tube [9]. Ectopic expression of BMPs interferes with development of the FP and MNs, and results in a ventral-to-dorsal shift of neuronal cell-subtype identity [10,11]. Thus, at least in the spinal cord, Shh probably requires the co-expression of BMP antagonists by the NC and FP.

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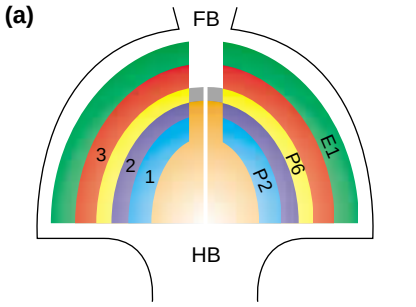

Fig. 2. Molecular patterning of the ventral mesencephalon. (a) The normal expression of ventral to lateral markers in the midbrain is nested from ventral to lateral around the midline expression of Sonic Hedgehog (Shh). (b) This panel shows the normal pattern of ventral midbrain markers (on left). Ectopic expression of Shh in lateral positions can result in a recapitulation of this pattern of gene expression (on right). Expression of Hx is shown in purple and FOXA2 in red. Reproduced, with permission from [12]. Abbreviations: FB, forebrain; HB, hindbrain.

In a recent study, Agarwala *et al.* provide the best *in vivo* evidence to date that a focal source of Shh can organize surrounding tissues in the chick CNS in a manner consistent with morphogen models of the function of Shh [12]. In the process, the authors demonstrate exquisite patterning in the mesencephalon in the form of 'arcs' of cellular expression patterns that delineate discrete dorsoventral domains. Although the authors focus on the role of Shh in establishing the arcs, rather than the organization of the mesencephalon itself, this work provides a glimpse of how patterning is established in this somewhat neglected division of the brain.

During embryonic development, the ventral midbrain is organized into five molecularly distinct arcuate territories arranged progressively more laterally to the ventral midline (Fig. 2a). The authors used *in vivo* electroporation of Shh cDNA at embryonic day 2 to generate ectopic sources of Shh of varying size and position, and then analyzed the outcome on midbrain patterning at embryonic day 5. Strikingly, they found that an ectopic point source of Shh expression in dorsolateral regions is sufficient to elicit a properly ordered set of the midbrain arcuate territories (Fig. 2b). Overall, the authors demonstrate convincingly that Shh can organize surrounding tissues in the midbrain, as predicted by the hypothesis that Shh acts as a morphogen.

Although the findings of this paper are consistent with a morphogen model of Shh signaling, the present results cannot distinguish whether the pattern generated by ectopic point sources of Shh results from long-range or relay signaling. Canonically, the binding of Shh to its receptor Patched (Ptc) alleviates repression of Smoothened (Smo), a transmembrane protein that transduces the Shh signal within the cell. Gain-of-function experiments using a constitutively active form of Smo did not yield any evidence for a relay model, in that all ectopic patterning appeared to be cell autonomous [13]. Furthermore, a recent study has shown that in the chick spinal cord, cells expressing a dominant-negative allele of Ptc fail to respond to Shh signaling and undergo a ventral to dorsal transformation even when adjacent to wild-type cells that have acquired ventral characteristics in response to Shh [14]. Thus, unless Shh functions through a dramatically different mechanism in the midbrain versus the spinal cord, it appears improbable that Shh signaling functions through a relay mechanism in either context.

Why, then, has the present study been successful in recapitulating ventral patterning with Shh alone, when previous studies have not? Perhaps the difference rests simply in the use of electroporation versus transgenic methods for the ectopic expression of Shh. Also, it might be that the BMP environment in the dorsolateral midbrain is more amenable to the induction of an ectopic ventral pattern than the dorsal spinal cord in that there is less BMP-mediated inhibition of ventral patterning to overcome. Regardless, the study by Agarwala *et al.* reveals that Shh functions in the ventral midbrain to establish an intricate developmental organization that has not been recognized previously. There is no doubt that the discovery of markers revealing this architecture will be essential to further teasing out answers to how patterning in this brain division is established.

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References

1 Jessell, T.M. (2000) Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nat. Rev. Genet.* 1, 20–29
2 Chiang, C. *et al.* (1996) Cyclopia and defective axial patterning in mice lacking Sonic Hedgehog gene function. *Nature* 383, 407–413
3 Ericson, J. *et al.* (1995) Sonic Hedgehog: a common signal for ventral patterning along the rostrocaudal axis of the neural tube. *Int. J. Dev. Biol.* 39, 809–816
4 Roelink, H. *et al.* (1995) Floor plate and motor neuron induction by different concentrations of the amino-terminal cleavage product of Sonic Hedgehog autoproteolysis. *Cell* 81, 445–455
5 Briscoe, J. and Ericson, J. (2001) Specification of neuronal fates in the ventral neural tube. *Curr. Opin. Neurobiol.* 11, 43–49
6 Briscoe, J. and Ericson, J. (1999) The specification of neuronal identity by graded Sonic Hedgehog signalling. *Semin. Cell. Dev. Biol.* 10, 353–362
7 Echelard, Y. *et al.* (1993) Sonic Hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* 75, 1417–1430
8 Yamada, T. *et al.* (1991) Control of cell pattern in the developing nervous system: polarizing activity of the floor plate and notocord. *Cell* 64, 635–647
9 Lee, K.J. and Jessell, T.M. (1999) The specification of dorsal cell fates in vertebrate central nervous system. *Annu. Rev. Neurosci.* 22, 261–294
10 Golden, J.A. *et al.* (1999) Ectopic bone morphogenic proteins 5 and 4 in the chicken forebrain lead to cyclopia and holoprosencephaly. *Proc. Natl. Acad. Sci. U. S. A.* 96, 2439–2444
11 Liem, K.F. *et al.* (2000) Regulation of the neural patterning activity of sonic hedgehog by secreted BMP inhibitors expressed by notocord and somites. *Development* 127, 4855–4866
12 Agarwala, S. *et al.* (2001) Sonic hedgehog control of size and shape in midbrain pattern formation. *Science* 291, 2147–2150
13 Hynes, M. *et al.* (2000) The seven-transmembrane receptor smoothened cell-autonomously induces multiple ventral cell types. *Nat. Neurosci.* 3, 41–46
14 Briscoe, J. *et al.* (2001) A hedgehog-insensitive form of patched provides evidence for direct long-range patterning of sonic hedgehog in the neural tube. *Mol. Cell* 7, 1–20

Robert Machold
Gord Fishell*
Developmental Genetics Program and the Dept of Cell Biology, The Skirball Institute of Biomolecular Medicine, NYU Medical Center, 540 First Avenue, NY 10016, USA.
*e-mail: fishell@saturn.med.nyu.edu