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Developmental genetics in Sheffield: a meeting point for Hedgehog researchers

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Situated at the edge of the picturesque Peak District (see Fig. 1) on the border between Derbyshire and Yorkshire and some 160 miles north of London, Sheffield is home to one of a group of Universities founded in the major industrial cities of England at the beginning of the 20th century. In the 1930s, the arrival of Hans Krebs from Freiburg established the University as a leading centre for biochemical research: it was in Sheffield that Krebs elucidated the TCA cycle which now bears his name and which is permanently engrained upon the minds of every biochemistry undergraduate across the world! The laboratories in which Krebs conducted his seminal research have more recently become home to a number of structural biologists with major research programmes that include the analysis of prion proteins and of protein-nucleic acid interactions.

In 1997, a new Developmental Genetics Programme was established at Sheffield University to complement the existing strengths in the biological and biomedical sciences. Situated adjacent to the Structural Biology group in the imposing Firth Court building, the Developmental Genetics Programme brings together researchers using invertebrate and vertebrate model systems to address a number of fundamental developmental processes. These range from the molecular mechanisms underlying axon guidance in the vertebrate CNS (see for example Cohen *et al.,* 1997) to the cellular basis of organogenesis in the *Drosophila* embryo (see for example Wan *et al.*, 2000): but a central theme linking all of the research within the Programme is the role of cell-cell interactions and the signalling pathways that mediate them. In recognition of this, several groups within the Programme have been accorded the status of a Co-operative Group by the UK Medical Research Council and funding from this agency, along with substantial support from the Wellcome Trust, underpins most of the activities within the Programme. Many of the intercellular signalling pathways familiar to developmental biologists impinge upon the research of the individual groups, but none more so than that of the Hedgehog family of signalling proteins. In what follows, we provide a brief account of studies performed over the last fifteen years on the mechanisms of Hedgehog signalling and the processes that it mediates. We highlight the ways in which our own particular research activities, which initially followed quite different paths, have subsequently converged, providing the foundation for the establishment of the Developmental Genetics Programme in Sheffield.

Abbreviations used in this paper: mABs, monoclonal antibodies; FP3, floorplate 3; FP4, floorplate 4; MRC, Medical Research Council; LMB, Laboratory of Molecular Biology; wg, wingless; en, engrailed; nkd, naked; ptc, patched; DNA, deoxyribonucleic acid; hh, hedgehog; HFSP, Human Frontier Science Program; Dhh, Desert hedgehog; ZPA, Zone of polarising activity; CNS, Central nervous system; TGFβ, Transforming growth factor beta; BMP, Bone morphogenic protein; MPs, Muscle pioneers; Ehh, Echidna hedgehog; fu, fused; ci, cubitus interruptus; cos-2, costal-2; Su(fu), Suppressor of fused; PKA, Protein kinase A; smo, smoothened.

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Fig. 1. Three views of the Peak District National Park, *about ten miles from the University.*

The vertebrate strand: 1985-1992

Much of our understanding of the role of Hedgehog genes in vertebrate patterning stems from work performed in New York in the labs of two British researchers, Tom Jessell and Jane Dodd. In the early 1980s, the focus of research in these labs was on the guidance of axons within the rat spinal cord. Jessell and Dodd had used the newly-emerging technique of monoclonal antibody production to identify a specialised group of cells located at the ventral midline of the neural tube. Decades earlier, in the 1920s, German scientists had in fact identified the same cells through their distinctive morphology, and provided them with a name –'boden platte', or 'floor plate'. Studies by Jessell and his colleagues, together with another British scientist, Andrew Lumsden working at Guy's Hospital Medical School in London, now revealed an important function of the floor plate, namely its ability to synthesise a chemoattractant for developing axons (Tessier-Lavigne *et al.*, 1988). One of the post-doctoral fellows at the centre of this work, Marc Tessier-Lavigne, went on to identify the chemoattractant as Netrin-1, a molecule critical to the guidance of embryonic axons (Colamarino and Tessier-Lavigne, 1995).

The availability of the newly isolated mABs against floor plate specific cell-surface markers –such as FP3 and FP4– now allowed one of us (MP), also at the time a post-doctoral fellow in the Jessell-Dodd groups, to address a question that had long been in dispute –that of the origin of floor plate cells. On the one hand was the idea that floor plate cells acquired their specialised properties because of their lineage. Fate mapping studies by Dale and Slack (1987) had revealed that a dorsally-located blastomere in the 32-cell stage *Xenopus* embryo would give rise to the floor plate and to a specialised mesodermal structure located immediately beneath it, the notochord. In the early gastrula embryo, the precursors of both cell types remain juxtaposed, in and adjacent to the organiser. The shared early lineage of notochord and floor plate, together with the discovery that they share antigenic determinants, thus provided support for the idea that the floor plate differed from other cells in the spinal cord because of its past history (Jessell *et al.*, 1989). At odds with this, however, were studies performed in chick embryos, suggesting that the acquisition of specialised properties by the floor plate was dependent upon an induction by the notochord (van Straaten *et al.*, 1988).

To distinguish between these possibilities, Placzek and colleagues performed *in vivo* experiments in chick embryos. The results were clear-cut: when fragments of notochord were grafted next to the neural tube, ectopic floor plate cells differentiated in the adjacent neural tube, indicating that notochord can induce floor plate (Fig. 2). Conversely, when fragments of notochord were removed from chick embryos, an experiment suggested by Claudio Stern (then at Oxford University), floor plate cells failed to develop in the overlying neural tube, showing the requirement for notochord in normal floor plate development (Placzek *et al.*, 1990, 2000; Yamada *et al.*, 1991). Critical to the subsequent development of these studies was a technique pioneered for the analysis of the chemoattractive properties of the floor plate, namely, the *in vitro* culture of tissues within 3-dimensional collagen gels (Fig. 3). Such cultures allowed a more rigorous assessment of the likely properties of the floor plate-inducing factor, and culminated in a model in which the notochord-derived factor acted at short range to induce floor plate cells. In addition, the model proposed that the induction was of a homeogenetic (like-begets-like) nature, such that newly-induced floor plate cells would themselves acquire floor plate-inducing ability (Placzek *et al.*, 1993).

Concurrent studies by Toshiya Yamada, in Jessell's lab, led to a further key realisation: in notochordectomised embryos, not only were floor plate cells missing, but in addition, motor neurons were not generated (Yamada *et al.*, 1991). This early observation subsequently fuelled a massive area of research, all of which has

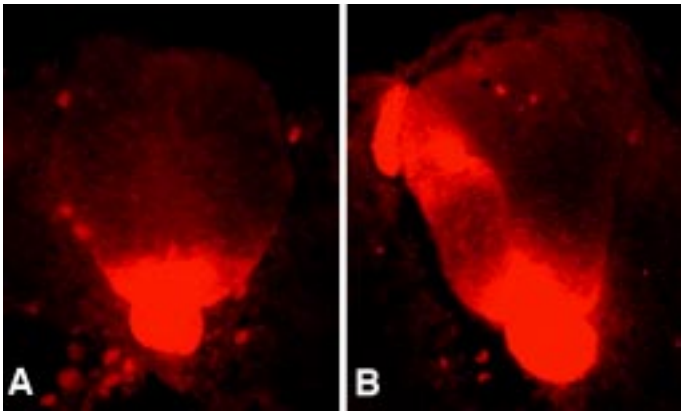

Fig. 2. Notochord graft induces Shh-expressing floor plate cells. (A) *Transverse section through an E2.5 chick spinal cord, labelled with anti-Shh antibody. Shh is detected in notochord cells and in overlying floor plate cells.* **(B)** *Transverse section through an E2.5 chick spinal cord, 24 h after a lateral notochord graft. Shh expression is detected on the host notochord and floor plate and on the grafted notochord. In addition, Shh expression is induced on ectopic floor plate cells immediately adjacent to the grafted notochord.*

revealed a shared early patterning function of the notochord and the floor plate, namely their ability to confer ventral identity on adjacent neural tissue, causing the differentiation of numerous cell types including motor neurons (Fig. 3) and ventral interneurons, (reviewed in Tanabe and Jessell, 1996).

By the early 1990s the importance of the notochord and floor plate in patterning neural tissue in the spinal cord and hindbrain was widely accepted. However, almost nothing was known about the molecular basis for their patterning functions. Did a single factor emanate from notochord and floor plate to mediate their patterning abilities, acting as a morphogen to elicit the differentiation of distinct cell types? Or did the notochord and floor plate synthesise an array of molecules, each with the ability to induce a distinct cell type? Was there any evidence that retinoids, thought to be present in the notochord and floor plate, and to act as morphogens in other systems, could mediate the effects of the notochord and floor plate? (Wagner *et al.*, 1990; Tickle and Eichele, 1994).

With the realisation that the phenomenological studies could not continue without an accompanying molecular understanding, all thoughts turned to ways in which the factors mediating the patterning functions of notochord and floor plate could be cloned. The breakthrough was to come from what, at the time, seemed a quite unexpected quarter: the isolation of homologues of a gene involved in patterning the larval segments of the fruit-fly *Drosophila*.

The *Drosophila* strand 1980-1992

The 1980s witnessed unprecedented progress in the molecular characterisation of pattern formation in *Drosophila*. This activity was fuelled largely by the many mutants isolated in the seminal screens of Nüsslein-Volhard and Wieschaus: these screens identified entire classes of hitherto unsuspected genes that act sequentially to subdivide the developing embryo into its final segmented form. Initial studies focused on the cascade of transcriptional activity triggered by maternally deposited factors and proceeding via the gap, pair rule, and segment polarity genes (reviewed in Ingham, 1988). But in 1986, working at the MRC LMB in Cambridge one of us (PI) together with Alfonso Martinez-Arias and Nick Baker, embarked upon the first large scale survey of the interactions *between* the segment polarity genes themselves. Using the only two molecular probes available at the time, Martinez-Arias *et al.* (1988) surveyed the expression of *wingless (wg)* and *engrailed (en)* in all of the extant segment polarity mutants. The results were intriguing, revealing a symmetry in the spatial regulation of the two genes: while most segment polarity genes appeared to be essential for the maintenance of expression of both *wg* and *en*, in two cases the opposite relationship seemed to hold. Thus in *naked (nkd)* mutants, the domain of *en* expression was found to be expanded posteriorly while in *patched (ptc)* mutants the domain of *wg* expression clearly broadened in an anterior direction (Fig. 4A). Moreover, in each instance the early expansion of one or other gene was followed by the *de novo* induction of expression of the complementary gene in immediately adjacent cells. Thus in *nkd* mutants expression of *en* is accompanied by ectopic *wg* expression in the cells adjacent to those expressing *en*, whilst in *ptc* mutants, expansion of *wg* expression is accompanied by ectopic *en* expression in the middle of each segment (Fig. 4B). This quite unexpected finding provided a key insight into the cell-cell interactions that underlie the patterning of each segment: taken together with the finding that *en* and *wg* mutually regulate one another, it suggested a model in which local cell-cell interactions specify the fates of individual cells within the segment. The almost contemporaneous discovery that *wg* encodes the *Drosophila* version of the secreted glycoprotein, Wnt-1 (Rijsewijk *et al.*, 1987), naturally, lent considerable weight to this model: extensive characterisation of Wnt-1 (or int-1 as it was still known at this time) had implicated it as a putative signalling molecule that acts at relatively short range. So the dependence of *en* expression on the activity of *wg* expressed in immediately adjacent cells suggested the appealing notion that Wg itself might mediate this local cell-cell interaction. How *en* might recipro-

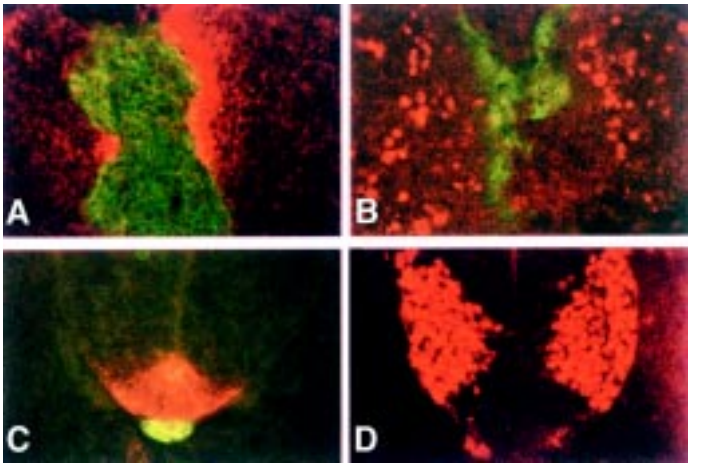

Fig. 3. Induction of floor plate and motor neurons *in vitro*. (A,B) *Serial adjacent sections of a notochord-lateral neural plate recombinate, double labelled to detect* **(A)** *the notochord marker 3B9 (green) and the floor plate marker, FP3 (red), or* **(B)** *3B9 and the motor neuron marker, Islet-1 (red). Floor plate cells differentiate in the neural tissue immediately adjacent to the notochord. Motor neurons differentiate at a distance from the notochord.* **(C)** *Transverse section through an E13 rat spinal cord. Notochord cells (labelled in green with anti-FP4) lie immediately beneath floor plate cells (labelled in red with anti-FP3).* **(D)** *Transverse section through an E13 rat spinal cord. Motor neurons, labelled with anti-Islet 1, differentiate at a distance from the notochord and floor plate.*

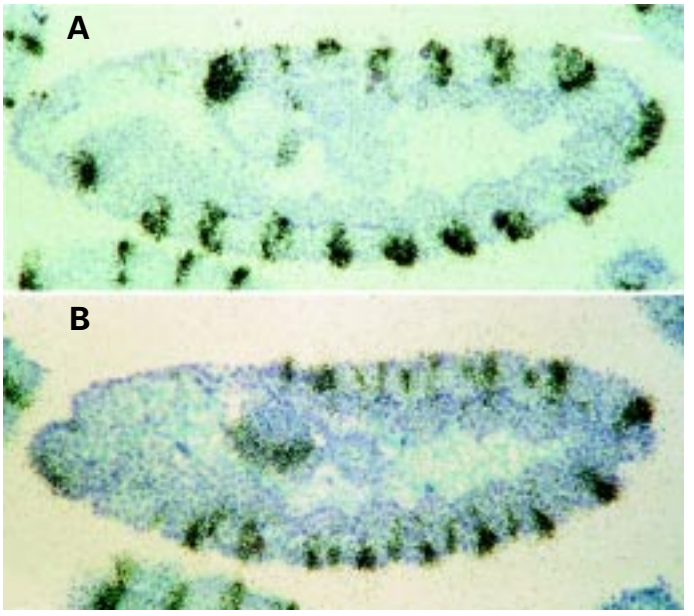

Fig. 4. Altered patterns of wg and en expression in *Drosophila* embryos mutant for patched. *Prior to the introduction of non-isotopic labelling techniques, visualising the expression of two different genes in the same embryo relied upon the hybridisation of adjacent histological sections with different probes. This figure shows an example of such analysis, in fact the very first stage 11 ptc mutant embryo to be analysed in this way! The upper panel* **(A)** *shows the broadened bands of wg expression typical of ptc mutants: the lower panel* **(B)** *shows the additional stripes of en expression that are induced in response to the ectopic wg.*

cally maintain the expression of *wg* was less clear-cut: since *en* encodes a homeoprotein, the most likely solution seemed to be that *en* regulates the expression of another short-range signalling molecule: but the identity of the gene that encodes this signal was not immediately obvious.

Shortly after this initial study was completed, Ingham moved to Oxford and embarked upon the molecular cloning of *ptc*. This choice of gene was influenced partly by the interesting role of *ptc* in regulating *wg* expression but also, more pragmatically, by the discovery by Robert Whittle at Sussex University, that *ptc* is allelic to a previously known homozygous viable mutant named *tufted*. Since *tufted* mutants cause an easily scored partial duplication of the wing, this discovery opened the way to identifying new mutations in *ptc* induced by P-element insertions. Following the isolation of a number of such alleles, the genomic DNA encoding *ptc* was cloned by Ingham and two Spanish colleagues in Oxford, Isabel Guerrero and Alicia Hidalgo. The analysis of *ptc* expression revealed an unexpectedly complex and highly dynamic pattern that at first served only to obscure its function (Nakano *et al.,* 1989). After a careful analysis of this pattern in both wild-type and segment polarity mutant embryos, however, a picture began to emerge that implicated one gene in particular, *hedgehog*, in somehow mediating the signalling from *en*-expressing cells to their immediately adjacent neighbours (Hidalgo and Ingham, 1990). Of key importance in reaching this conclusion was the observation that towards the end of the germ band extension stage, the expression of *ptc* becomes up-regulated in two narrow stripes of cells that flank the *en* expression domain, one of which accordingly coincides with *wg* expression. Crucially, both stripes of *ptc* expression as well as the single stripe of *wg* in each segment were found to disappear in *hh* mutant embryos. Given the recently published findings of Mohler (1988) that *hh* acts in a non-autonomous fashion in the developing wing, these observations strongly suggested that *hh* may encode the signal that mediates *en*-dependent cell-cell interactions. The most parsimonious explanation of the data thus seemed to be that *en*-expressing cells secrete Hh which acts on either side of the *en* domain to regulate the fate of neighbouring cells. Direct evidence for such a role of *hh* was not to come for a further two years when the *hh* gene was cloned independently by four different groups (Lee *et al*., 1992; Mohler and Vani, 1992; Tabata *et al*., 1992; Tashiro *et al*., 1993): this allowed the generation of antibodies (Taylor *et al*., 1993; Tabata and Kornberg, 1994) that showed conclusively that Hh is secreted by *en*-expressing cells (see Fig. 5).

In the meantime, the story was to take a further twist with the implication of *ptc* as the receptor for the signal putatively encoded by *hh*. The cloning of *ptc* had revealed that it encodes a multi-transmembrane protein yet its functional analysis suggested that it acts to repress transcription of *wg*. How could these two properties of the same gene product be reconciled? The solution lay in the epistasis analysis of *hh* and *ptc*: this revealed that in the absence of *ptc* activity, *wg* expression not only expands but becomes independent of *hh* activity. This finding led directly to the suggestion that *ptc* activity suppresses *wg* transcription in all cells that are competent to express *wg* in response to Hh: in cells that receive Hh, the activity of Ptc is antagonised, allowing *wg* to be transcribed (Ingham *et al*., 1991). Since Hh was postulated to be a secreted protein and Ptc had been shown to be a membrane spanning protein, the simplest way in which this could be achieved seemed to be by Hh binding to Ptc. Although this model met with much scepticism at the time, direct biochemical evidence for such an interaction between Ptc and Hh proteins was published in 1996 (Marigo *et al*., 1996; Stone *et al*., 1996) and the model is now generally accepted. Further evidence for the functional interaction between Ptc and Hh was subsequently adduced by the analysis of both *wg* and *ptc* transcription in various mutant combinations involving *hh* and pair-rule genes (Ingham and Hidalgo, 1993). These studies –that coincidentally were published in the same issue of *Development* as Placzek *et al's* (1993) study of contact dependent induction of FP by the notochord– reinforced the notion of a regulatory relationship between Hh and Ptc and emphasised the essentially short-range nature of the Hh signal.

While the story of *wg* regulation by *ptc* and *hh* in *Drosophila* had been unfolding, studies of the vertebrate *Wnt-1* and *En* genes had revealed some remarkable similarities in their spatial deployment in the vertebrate CNS. Although not expressed in the familiar metameric pattern of the *Drosophila* genes, *Wnt-1* and *En-2* had been shown to be expressed in close association with one another at a very significant location –the boundary between the mid brain and hindbrain. Moreover, the evolution of *Wnt-1* expression from an initially broad domain to an ultimately narrow stripe abutting the *En*-expressing hindbrain, recalled the highly restricted expression of *wg* adjacent to *en*-expressing cells in each embryonic segment in *Drosophila*. Could it be that a *hh* gene, under the transcriptional control of *En-2*, might mediate the maintenance of *Wnt-1* expression at the midbrain-hindbrain boundary? With this question in mind Ingham spent a warm summer's day in 1992 in Oxford with Andrew McMahon (of Harvard University) drafting a proposal to the Human Frontiers of Science Programme (HFSP) to clone homo-

logues of *hedgehog* from zebrafish and mouse. Before the proposal was submitted, Cliff Tabin, from Harvard Medical School was added as a collaborator (see Fig. 6): the inclusion of Tabin, an expert on chick limb development, was to prove of crucial significance in what followed.

The initial results of the cloning project seemed disappointing: expression of the first gene to be isolated, by McMahon's laboratory, was, contrary to expectation, conspicuously absent from the developing embryo, though it was detectable in the adult testis. Using this gene –subsequently named *Dhh*– as a probe, Stefan Krauss in P. Ingham's lab in Oxford screened a zebrafish cDNA library and isolated a single clone. Given the results of the *Dhh* expression analysis in mouse, Krauss' appetite for performing *in situ* hybridisations with this probe had been significantly diminished. However, the news from the Tabin laboratory that they had succeeded in using PCR to identify three distinct *hh*-related sequences from the chick stimulated renewed enthusiasm. Krauss immediately performed the *in situs* with his clone –and the results were quite remarkable. Unlike *Dhh* the zebrafish *hh* homologue was very obviously expressed during embryogenesis, but not in the mid-hind brain boundary as we had expected: rather this gene –soon to become known as *Sonic hedgehog*– was found to be expressed throughout the axial mesoderm and in the ventral region of the overlying neural tissue (see Fig. 7). These sites of expression –and most notably the notochord and floorplate– were striking not least because of the implication of these tissues in the inductive interactions that pattern the neural tube described above. Moreover, Tabin's group quickly established that *Shh* is also expressed in a restricted region of each limb bud, corresponding precisely to the operationally defined zone of polarising activity (ZPA). In the 1960s and 70s it had been found that grafting of this small piece of posterior mesenchyme to ectopic sites within the limb bud could elicit the duplication of distal elements of the limb –notably the digits– in a pattern with inverse polarity to the normal digits (reviewed in Tickle and Eichele, 1994). This had led to the suggestion that the ZPA might act as a source of a secreted signalling molecule that patterns the developing limb. So could this signalling molecule be the product of the *Shh* gene? The fact that notochord and floorplate were known to mimic the effects of the ZPA in the grafting experiments (Wagner *et al.,* 1990) made this possibility seem all the more likely. Within weeks of isolating the clone, Tabin's lab had constructed a retroviral expression construct that allowed *Shh* to be misexpressed in the developing limb –the results were extraordinary: ectopic digits developed with inverse polarity, just as in the case of a ZPA graft (Riddle *et al.*, 1993). These data, together with the ectopic induction of ventral markers following misexpression of *Shh* in the CNS of the zebrafish (Krauss *et al.*, 1993) and mouse (Echelard *et al.*, 1993), strongly implicated Shh as a signalling molecule that mediates the effects of both the ZPA and the notochord and floor plate, a conclusion supported by *in vitro* induction studies (Roelink *et al.,* 1994).

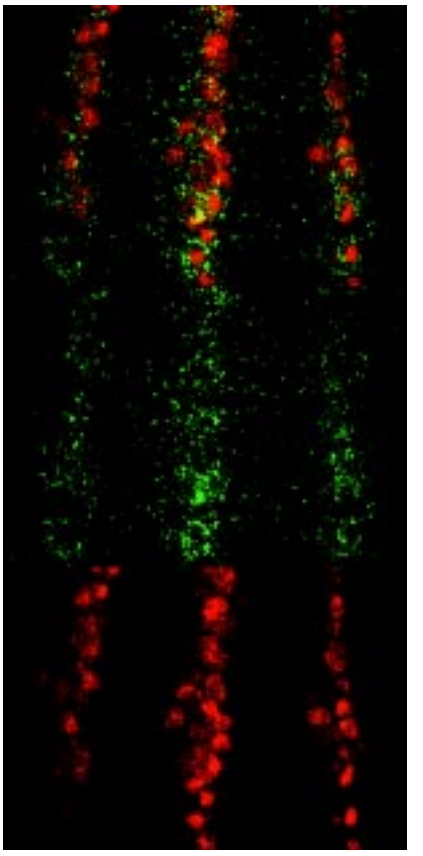

Fig. 5. Hh protein is secreted by En expressing cells at the anterior margin of each parasegment in the *Drosophila* embryo. *The lower two panels show Hh protein in green and En protein in red in three adjacent parasegments of an early stage 10 wild-type Drosophila embryo. The merged images (top panel) reveal that the Hh protein distribution is centred on the En expressing cells (which transcribe the hh gene): note however that Hh protein moves away from the cells from which it is secreted, as revealed by the punctate pattern of labelling between the En domains.*

Sonic hedgehog in neural patterning

In 1996, Beachy and colleagues described the generation of a null mutant allele of Shh in the mouse, analysis of which confirmed a role for the gene in floor plate development (Chiang *et al.*, 1996). Addressing the question of whether, and how, Shh can mediate the induction of multiple cell types along the dorso-ventral axis of the neural tube, however, has depended upon the ability to express and purify recombinant Sonic hedgehog protein. Numerous *in vitro* analyses over the past three or four years have provided compelling evidence that Shh does indeed function as a morphogen, the differentiation of distinct cell types occurring in response to different threshold concentrations of the protein (Tanabe and Jessell, 1996; Ericson *et al.*, 1997). The competence of the neural tube to respond to Shh appears also to alter over time. In the early spinal cord, neural progenitors respond to Shh by assuming the fate of motor neurons and ventral interneurons; later in embryogenesis, precursor cells in the ventral neural tube differentiate in response to Shh and assume an oligodendrocyte fate (Orentas *et al.,* 1999). Thus, Shh operates in conjunction with other determinants that restrict and alter the outcome of its signalling activity.

The capacity of other factors to impinge upon and alter the outcome of Shh signalling, appears critical to the development of forebrain ventral midline cells. A key concept that emerged upon the cloning and expression analysis of *Shh* was that its profile in ventral midline cells did not precisely match the expression profile of the various markers of floor-plate cells (such as FP3 and FP4). Thus, whereas antigenic and functional analyses show that floor plate cells extend only through the spinal cord, hind- and mid-brain, Shh expression extends into a group of ventral midline cells located in the forebrain (see Fig. 7). Expression of Sonic hedgehog within these cells appears to play a similar patterning role to that in more posterior domains of the central nervous system: studies of mutant zebrafish embryos lacking this domain of Shh expression, suggested that the forebrain ventral midline patterns the adjacent neuroepithelium, including ventral regions of the prospective eye (Krauss *et al.*, 1993; Ecker *et al.,* 1995; Macdonald *et al.,* 1995).

Fig. 6. The first meeting of the HFSP funded Hedgehog cloning consortium on the roof of the Department of Zoology in Oxford, 1993. *From left to right: Tom Schilling (I), Randy Johnson (T), Philip Ingham, David Simmons (Oxford colleague of PI), Andy McMahon, Jean-Paul Concordet (I), Cliff Tabin, R. Takad (M), Uwe Strähle (I), Valerio Marigo (T), David Rowitch (M), Doug Epstein (M), Ed Laufer (T) and Bob Riddle (T). Stefan Krauss, who cloned Shh from zebrafish, arrived in Oxford shortly after this photograph was taken. Letters in parentheses indicate lab affiliation (I): Ingham; (M) McMahon; (T) Tabin.*

A key question that emerges then, is why Shh expression in forebrain ventral midline cells is not accompanied by expression of floor plate characteristics. Studies by Placzek and her colleagues suggested that this discrepancy arises due to a conjunction of Shh signalling and signalling by the TGFβ superfamily member, bone morphogenetic protein 7 (BMP7). In the forebrain, ventral midline cells are induced by underlying axial mesoderm cells of the prechordal mesoderm. In contrast to notochord cells, prechordal mesoderm cells co-express Shh and BMP7. *In vitro* studies suggest that the expression of BMP7 by prechordal mesoderm alters the outcome of Shh signalling, such that forebrain ventral midline cells are induced, instead of floor plate cells (Dale *et al.*, 1997). How these two signalling pathways operate in concert to govern the differentiation of forebrain ventral midline cells is currently a major focus of current research.

Sonic hedgehog in mesodermal patterning

In addition to its key contribution to the ventralisation of neural tissues, the potential role of Shh in mediating inductive interactions between the notochord and the flanking paraxial mesoderm was soon recognised and investigated. At first, studies suggested a role for Shh in specifying the sclerotomal compartment of each somite (Fan *et al.*, 1995), but evidence for an involvement in inducing the myotome also quickly accumulated (Johnson *et al.*, 1994; Munsterberg and Lassar, 1995). The initial picture, based largely upon *in vitro* and *in vivo* manipulation of the chick embryo, was complicated by the finding that elimination of Shh activity from the mouse embryo does not lead to a complete loss of the myotome (Chiang *et al.*, 1996). Careful analysis of the Shh knock-out mouse by Anne-Gaelle Borycki, now in Sheffield but at the time working with Charles Emerson at the University of Pennsylvania, subsequently revealed that Shh is required specifically for the induction of the epaxial component of the myotome (Borycki *et al.*, 1999). By analysing the expression of the GLI transcription factors that mediate Shh signalling, Borycki and colleagues (1998) found that the ability to respond to Shh becomes limited to a subset of cells within each somite. Thus, as in the neural plate, the effects of Hh signalling depend not only upon the levels and range of the signal but also upon the competence of cells to respond to it.

Studies in the Ingham laboratory have revealed a similar interaction between the notochord and paraxial mesoderm in the zebrafish embryo: in this case, however, the interaction appears to be mediated not just by Shh but by multiple members of the Hh family. The first clue to this came from the finding that a specialised

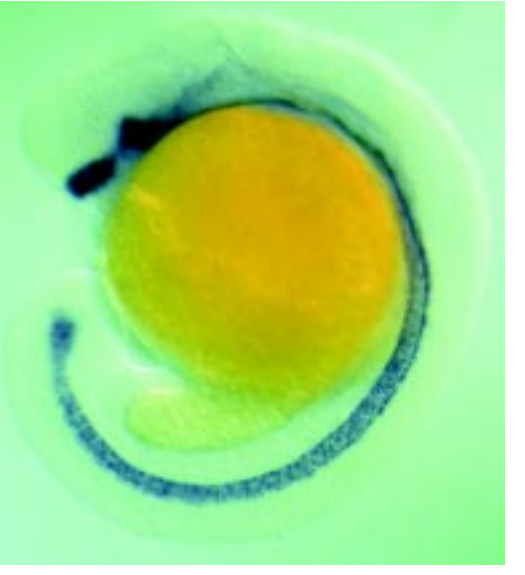

Fig. 7. *Shh* is expressed in midline structures in the developing vertebrate embryo. *The pattern of Shh expression in the notochord and ventral floor of the nervous system was first seen in the zebrafish embryo where it is particularly striking, thanks to the optical clarity of the material. This example shows an embryo at the 16-20 somite stage when the notochord expression is still very prominent. Note also that Shh expression extends into the ventral floor of the forebrain.*

group of cells –the muscle pioneers (MPs)– fail to differentiate in mutants that express Shh but lack the expression of another Hh family gene, Echidna hedgehog (Ehh), which is normally expressed by the notochord of fish (Currie and Ingham, 1996). MPs derive from cells that initially lie adjacent to the notochord and give rise to all the slow-twitch muscle cells of the embryo. Subsequent studies have shown that these so-called adaxial cells depend upon the combined activities of the midline expressed Hh genes for their induction. Removal of Shh activity alone (by mutation) reduces the number of slow muscle cells that differentiate and eliminates all MPs, while blocking the signalling pathway downstream of the receptor (through mutation of the transcription factor *gli2*) completely eliminates the induction of all slow muscle (Lewis *et al.,* 1999). Current studies in the Ingham laboratory aim at understanding how MPs and other slow muscle cell identities come to be induced by different levels of the various Hh proteins and at characterising the genes that control the response of cells to these signals.

Unravelling the Hh signalling pathway

Remarkably, and uniquely, all bar one of the components of the Hh signalling pathway identified to date have been discovered through studies in *Drosophila.* Using genetic epistasis analysis to dissect the control of *hh*-dependent *ptc* and *wg*, Ingham and colleagues (Forbes *et al.,* 1993; Ingham, 1993) established the basic genetic pathway that has informed subsequent elucidation of Hh signal transduction at the molecular and biochemical levels. According to these studies, the products of the segment polarity genes *fused (fu)* and *cubitus interruptus (ci)* together with a third gene, *costal-2 (cos-2)*, all act downstream of *hh* and *ptc* to regulate the expression of *hh*-target genes. This model was soon after elaborated by characterisation of the *Su(fu)* gene (Préat *et al*., 1993), and through the discovery, by another member of the Developmental Genetics Programme, David Strutt, at the time a post-doctoral fellow at the EMBL, that Protein Kinase A (PKA) acts as a negative regulator of Hh target gene expression (Strutt *et al*., 1995) –a discovery that was made simultaneously and independently in four other laboratories around the world. The first molecular evidence for a direct involvement of any of these genes in Hh signalling came in 1996 with the demonstration that the Ci protein is a transcriptional activator that binds upstream of the *ptc* promoter to regulate its expression by Hh (Alexandre *et al*., 1996). Subsequent biochemical studies have revealed that Ci activity is regulated at several levels including phosphorylation, cleavage and nuclear shuttling, processes that depend upon interactions between Ci and the Fu, Cos-2 and Su(fu) proteins (reviewed by Ruiz i Altaba, 1999).

The identification of human Ptc as a tumour suppressor gene (Hahn *et al*., 1996; Johnson *et al*., 1996) provided the first direct evidence for an involvement of the Hh signalling pathway in tumorigenesis. The cloning and characterisation of another segment polarity gene *smoothened (smo)* the same year added a further twist to the story and led to the currently accepted view of the Hh receptor as a complex formed by the products of these two genes: a signalling subunit, encoded by *smo* and a regulatory component encoded by *ptc* (Murone *et al.,* 1999). Consistent with this view, oncogenic forms of Smo have subsequently been isolated from human tumour cell lines (Xie *et al.,* 1998). This link between Hh and oncogenesis has added a new dimension to our appreciation of the importance of this unusual signalling system, proving once again the value of fundamental research in developmental biology.

KEY WORDS: *hedgehog, patched, segment polarity, floor plate, induction, myotome*

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References

ALEXANDRE, C., JACINTO, A. and INGHAM, P.W. (1996). Transcriptional activation of *hedgehog* target genes in *Drosophila* is mediated directly by the Cubitus interruptus protein, a member of the GLI family of zinc finger DNA-binding proteins. *Genes Dev. 10:* 2003-2013.

BORYCKI, A., BRUNK, B., TAJBAKHSH, S., BUCKINGHAM, M., CHIANG, C. and EMERSON, C. (1999). Sonic hedgehog controls epaxial muscle determination through *myf5* activation. *Development 126:* 4053-4063.

BORYCKI, A.-G., MENDHAM, L. and EMERSON Jr, C. (1998). Control of somite patterning by Sonic hedgehog and its downstream signal response genes. *Development 125:* 777-790.

CHIANG, C., LITINGTUNG, Y., LEE, E., YOUNG, K.E., CORDEN, J.L., WESTPHAL, H., and BEACHY, P.A. (1996). Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature 383*: 407-413.

COHEN, N.R., TAYLOR, J., SCOTT, L., GUILLERY, R., SORIANO, P. and FURLEY, A. (1997). Errors in corticospinal axon guidance in mice lacking the neural cell adhesion molecule L1. *Curr. Biol. 8:* 26-33.

COLAMARINO, S.A. and TESSIERLAVIGNE, M. (1995). The role of the floor plate in axon guidance. *Annu. Rev. Neurosci. 18:* 497-529.

CURRIE, P.D. and INGHAM, P.W. (1996). Induction of a specific muscle cell type by a novel hedgehog gene family member. *Nature 382:* 452-455.

DALE, J.K., VESQUE, C., LINTS, T.J., SAMPATH, T.K., FURLEY, A., DODD, J. and PLACZEK, M. (1997). Cooperation of BMP7 and SHH in the induction of forebrain ventral midline cells by prechordal mesoderm. *Cell 90:* 257-269.

DALE, L. and SLACK, J. (1987). Fate map for the 32-cell stage of *Xenopus* laevis. *Development 99:* 527-551.

ECHELARD, Y., EPSTEIN, D.J., STJACQUES, B., SHEN, L., MOHLER, J., MCMAHON, J.A. and MCMAHON, A.P. (1993). Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell 75:* 1417-1430.

ECKER, S., UNGAR, A.R., GREENSTEIN, P., VON KESSLER, D.P., PORTER, J.A., MOON, R.T. and BEACHY, P.A. (1995). Patterning activities of vertebrate hedgehog proteins in the developing eye and brain. *Curr. Biol. 5:* 944-955.

ERICSON, J., BRISCOE, J., RASHBASS, P., VAN HEYNINGEN, V. and JESSELL, T.M. (1997). Graded sonic hedgehog signaling and the specification of cell fate in the ventral neural tube. *Cold Spring Harbor Symp. Quant. Biol. 62:* 451-466.

FAN, C.M., PORTER, J.A., CHIANG, C., CHANG, D.T., BEACHY, P.A. and TESSIERLAVIGNE, M. (1995). Long-range sclerotome induction by sonic hedgehog: direct role of the amino-terminal cleavage product and modulation by the cyclic AMP signaling pathway. *Cell 81:* 457-465.

FORBES, A.J., NAKANO, Y., TAYLOR, A.M. and INGHAM, P.W. (1993). Genetic analysis of *hedgehog* signalling in the *Drosophila* embryo. *Development (Suppl.):* 115-124.

HAHN, H., WICKING, C., ZAPHIROPOULOS, P.G., GAILANI, M.R., SHANLEY, S., CHIDAMBARAM, A., VORECHOVSKY, I., HOLMBERG, E., UNDEN, A.B., GILLIES, S., NEGUS, K., SMYTH, I., PRESSMAN, C., LEFFELL, D.J., GERRARD, B., GOLDSTEIN, A.M., DEAN, M., TOFTGARD, R., CHENEVIXTRENCH, G., WAINWRIGHT, B. and BALE, A.E. (1996). Mutations of the human homolog of *Drosophila patched* in the nevoid basal-cell carcinoma syndrome. *Cell 85*: 841-851.

HIDALGO, A. and INGHAM, P. (1990). Cell patterning in the *Drosophila* segment: spatial regulation of the segment polarity gene *patched. Development 110:* 291-302.

INGHAM, P.W. (1988). The molecular genetics of embryonic pattern formation in *Drosophila. Nature 335:* 25-34.

INGHAM, P.W. (1993). Localised *hedgehog* activity controls spatially restricted transcription of *wingless* in the *Drosophila* embryo. *Nature 366:* 560-562.

INGHAM, P.W. and HIDALGO, A. (1993). Regulation of *wingless* transcription in the *Drosophila* embryo. *Development 117:* 283-291.

INGHAM, P.W., TAYLOR, A.M. and NAKANO, Y. (1991). Role of the *Drosophila patched* gene in positional signalling. *Nature 353:* 184-187.

JESSELL, T.M., BOVOLENTA, P., PLACZEK, M., TESSIER-LAVIGNE, M. and DODD, J. (1989). Polarity and patterning in the neural tube: the origin and function of the floor plate. *CIBA Found. Symp. 144:* 290-295.

JOHNSON, R.L., LAUFER, E., RIDDLE, R.D. and TABIN, C. (1994). Ectopic expression of Sonic hedgehog alters dorsal-ventral patterning of somites. *Cell 79:* 1165-1173.

KRAUSS, S., CONCORDET, J.P. and INGHAM, P.W. (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.

LEE, J.J., VON KESSLER, D.P., PARKS, S. and BEACHY, P.A. (1992). Secretion and localised transcription suggests a role in oositional signalling for products of the segmentation gene hedgehog. *Cell 70:* 777-789.

LEWIS, K.E., CURRIE, P.D., ROY, S., SCHAUERTE, H., HAFFTER, P. and INGHAM, P.W. (1999). Control of muscle cell type specification in the zebrafish embryo by hedgehog signalling. *Dev. Biol. 216:* 469-480.

MACDONALD, R., BARTH, K.A., XU, Q., HOLDER, N., MIKKOLA, I. and WILSON, S.W. (1995). Midline signalling is required for Pax gene regulation and patterning of the eyes. *Development 121*: 3267-3278.

MARIGO, V., DAVEY, R., ZUO, Y., CUNNINGHAM, J. and TABIN, C. (1996). Biochemical-evidence that Patched is the Hedgehog receptor. *Nature 384:* 176-179.

MARTINEZ-ARIAS, A., BAKER, N.E. and INGHAM, P.W. (1988). Role of segment polarity genes in the definition and maintenance of cell states in the *Drosophila* embryo. *Development 103:* 157-170.

MOHLER, J. (1988). Requirements for *hedgehog*, a segment polarity gene, in patterning larval and adult cuticle of *Drosophila. Genetics 120:* 1061-1072.

MOHLER, J. and VANI, K. (1992). Molecular organisation and embryonic expression of the hedgehog gene involved in cell-cell communication in segmental patterning in *Drosophila. Development 115:* 957-971.

MUNSTERBERG, A.E. and LASSAR, A.B. (1995). Combinatorial signals from the neural tube, floor plate and notochord induce myogenic bHLH gene expression in the somite. *Development 121:* 651-660.

MURONE, M., ROSENTHAL, A. and DE SAUVAGE, F. (1999). Sonic hedgehog signaling by the patched-smoothened receptor complex. *Curr. Biol. 9:* 76-84.

NAKANO, Y., GUERRERO, I., HIDALGO, A., TAYLOR, A.M., WHITTLE, J.R.S. and INGHAM, P.W. (1989). The *Drosophila* segment polarity gene *patched* encodes a protein with multiple potential membrane spanning domains. *Nature 341:* 508-513.

ORENTAS, D.M., HAYES, J.E., DYER, K. and MILLER, R.H. (1999). Sonic hedgehog signaling is required during the appearance of spinal cord oligodendrocyte precursors. *Development 126:* 2419-2429.

PLACZEK, M., JESSELL, T.M. and DODD, J. (1993). Induction of floor plate differentiation by contact-dependent, homeogenetic signals. *Development 117:* 205-218.

PLACZEK, M., TESSIER LAVIGNE, M., YAMADA, T., JESSELL, T. and DODD, J. (1990). Mesodermal control of neural cell identity: floor plate induction by the notochord. *Science 250:* 985-988.

PLACZEK, M., DODD, J. and JESSELL, T.M. (2000). The case for floor plate induction b y the notochord. *Curr. Opin. Neurobiol.* (In press).

PRÉAT, T., THÉROND, P., LIMBOURG-BOUCHON, B., PHAM, A., TRICOIRE, H., BUSSON, D. and LAMOUR-ISNARD, C. (1993). Segmental polarity in *Drosophila melanogaster*: Genetic dissection of *fused* in a *Suppressor of fused* background reveals interaction with *costal-2. Genetics 135:* 1047-1062.

RIDDLE, R.D., JOHNSON, R.L., LAUFER, E. and TABIN, C. (1993). Sonic hedgehog mediates the polarizing activity of the ZPA. *Cell 75:* 1401-1416.

RIJSEWIJK, F., SCHUERMANN, M., WAGENAAR, E., PARREN, P., WEIGEL, D. and NUSSE, R. (1987). The *Drosophila* homolog of the mouse mammary oncogene *int-1* is Identical to the segment polarity gene *wingless. Cell 50:* 649-657.

ROELINK, H., AUGSBURGER, A., HEEMSKERK, J., KORZH, V., NORLIN, S., RUIZ I ALTABA, A., TANABE, Y., PLACZEK, M., EDLUND, T., JESSELL, T.M. and DODD, J. (1994). Floor plate andmotor neuron induction by vhh-1, a vertebrate homolog of hedgehog expressed by the notochord. *Cell 76:* 761-775.

RUIZ I ALTABA, A. (1999). The works of GLI and the power of Hedeghog. *Nature Cell Biol. 1:* E147-148.

STONE, D.M., HYNES, M., ARMANINI, M., SWANSON, T.A., GU, Q.M., JOHNSON, R.L., SCOTT, M.P., PENNICA, D., GODDARD, A., PHILLIPS, H., NOLL, M., HOOPER, J.E., DESAUVAGE, F. and ROSENTHAL, A. (1996). The tumor-suppressor gene *patched* encodes a candidate receptor for Sonic hedgehog. *Nature 384:* 129-134.

STRUTT, D.I., WIERSDORFF, V. and MLODZIK, M. (1995). Regulation of furrow progression in the *Drosophila* eye by cAMP- dependent protein kinase A. *Nature 373:* 705-709.

TABATA, T. and KORNBERG, T.B. (1994). Hedgehog is a signalling protein with a key role in patterning *Drosophila* imaginal discs. *Cell 76:* 89-102.

TABATA, T., EATON, S. and KORNBERG, T.B. (1992). The *Drosophila hedgehog* gene is expressed specifically in posterior compartment cells and is a target of *engrailed* regulation. *Genes Dev. 6:* 2635-2645.

TANABE, Y. and JESSELL, T.M. (1996). Diversity and pattern in the developing spinal cord. *Science 274:* 1115-1123.

TASHIRO, S., MICHIUE, T., HIGASHIJIMA, S., ZENNO, S., ISHIMARU, S., TAKAHASHI, F., ORIHARA, M., KOJIMA, T. and SAIGO, K. (1993). Structure and expression of *hedgehog*, a *Drosophila* segment-polarity gene required for cell-cell communication. *Gene 124:* 183-189.

TAYLOR, A.M., NAKANO, Y., MOHLER, J. and INGHAM, P.W. (1993). Contrasting distributions of patched and hedgehog proteins in the *Drosophila. Mech. Dev. 43:* 89-96.

TESSIER-LAVIGNE, M., PLACZEK, M., LUMSDEN, A.G.S., DODD, J. and JESSELL, T.M. (1988). Chemotropic guidance of developing axons in the mammalian central nervous system. *Nature 336:* 775-778.

TICKLE, C. and EICHELE, G. (1994). Vertebrate limb development. *Annu. Rev. Cell. Biol. 10:* 121-52.

VAN STRAATEN, H.M.W., HEKKING, J.M.W., WIERTZ-HOESSELS, E.L., THORS, F. and DRUKKER, J. (1988). Effect of the notochord on the differentiation of a floor plate area in the neural tube of the chick embryo. *Anat. Embryol. 177:* 317-324.

WAGNER, M., THALLER, C., JESSELL, T.M. and EICHELE, G. (1990). Polarizing activity and retinoid synthesis in the floor plate of the neural tube. *Nature 345:* 819-822.

WAN, S., CATO, A-M. and SKAER, H. (2000). Multiple signalling pathways establish cell fate and number in *Drosophila* Malpighian tubules. *Dev. Biol. 217:* 153-165.

XIE, J.W., MURONE, M., LUOH, S.M., RYAN, A., GU, Q.M., ZHANG, C.H., BONIFAS, J.M., LAM, C.W., HYNES, M., GODDARD, A., ROSENTHAL, A., EPSTEIN, E.H. and DESAUVAGE, F.J. (1998). Activating smoothened mutations in sporadic basal-cell carcinoma. *Nature 391:* 90-92.

YAMADA, T., PLACZEK, M., TANAKA, H., DODD, J. and JESSELL, T.M. (1991). Control of cell pattern in the developing nervous system: Polarizing activity of the floor plate and notochord. *Cell 64:* 635-647.