Vive la science! Vive le hérisson!

Jeremy F. Reiter & Frederic J. de Sauvage

The EMBO workshop 'Hedgehog Signalling: from Developmental Biology to Anti-cancer Drugs' took place between 27 and 31 March on the beautiful Côte d'Azur. The gathered scientists tackled topics ranging from the mechanisms by which Hedgehog (Hh) is presented to receptive cells, to the distribution of Hh by Glypicans, to the role of cilia in vertebrate Hh signal transduction, and to the function of Hh signals in cancer.

In the south of France, Pascal Théron, Alberto Gulino, Isabel Guerrero, Ariel Ruiz í Altaba and Rune Toftgård convened a conclave of Hedgehog (Hh) biologists to wander the rocky beaches and discuss new ideas about Hh signalling. Between doses of biology, we visited the *palazzo* of Baroness Béatrice Ephrussi. The Baroness, a scion of the Rothschild family, built a grand Venetian villa on Cap Ferrat. On recovering from a year-long illness, the Baroness apparently shed societal constraints, as she divorced her banker husband, cultivated a penchant for gambling, took up with a series of women, and threw parties for artists and high society. Béatrice Ephrussi also devoted herself to expansive gardens and a menagerie of monkeys, mongoose, budgies, antelopes and gazelles. Whether any *hérissons* (hedgehogs) were in residence is lost to the ages.

Within sight of her villa, comparably rarefied proceedings occupied the 2010 EMBO Hh workshop. The Baroness' *joie de vivre* infused the meeting, as best evidenced by Tom Kornberg's performance of Bach suites for unaccompanied cello. The scientific discussions similarly electrified the attendees, a few themes of which are described in this report.

Despite the field having reached a degree of maturity in the quarter century since the discovery of the first *Hh* gene, many fundamental questions remain about how Hh proteins signal and what those signals do. It does seem that the more one knows about this unintuitive signalling system, the more fascinating questions arise. Answering many of these questions requires combinations of cell biological, biochemical, structural and genetic approaches, and it is not clear that one answer need exclude other possibilities.

One question that remains an active focus of investigation is how the biologically relevant pool of Hh proteins is presented to responding cells. The endogenous Hh proteins are post-translationally modified with cholesterol and palmitate; yet, despite these hydrophobic moieties, Hh proteins can activate their signalling pathways many cell diameters away from the cell bodies of the producing cells. A focus of inquiry remains the cell biological and biochemical mechanisms by which Hh proteins communicate information to these receptive fields. Suzanne Eaton (MPI, Dresden) presented data indicating that Hh is distributed by lipoprotein particles (Fig 1). Lipoprotein particles are known mostly for their roles in transporting lipids and cholesterol in the vertebrate serum. Previously, Eaton and colleagues had demonstrated that *Drosophila* Hh can be associated with Lipophorin, a *Drosophila* lipoprotein, in low-density particles present in the imaginal disc space (Panakova *et al*, 2005). She described work showing that, similarly, HeLa cells provided with fetal calf serum also produce secreted SHH—a vertebrate Hh homologue—associated with lipoprotein particles. Extending the similarity between *Drosophila* and vertebrate lipoprotein particles, *Drosophila* Hh can associate with lipoproteins in the haemolymph, the serum equivalent in the fly.

What are the functions of circulating Hh-containing lipoprotein particles? In the absence of Hh, in addition to the well-described patterning defects, *Drosophila* imaginal discs show profound growth retardation. Eaton demonstrated that expressing Hh in the gut can partly rescue imaginal disc size. Thus, the circulating

form of Hh can signal—either directly in imaginal discs or indirectly through other tissues—to promote tissue growth. It will be interesting to determine whether the gut is the source of circulating Hh and whether haemolymph-borne Hh-containing lipoprotein particles are required for the growth of larval tissues. It will also be interesting to resolve whether lipoproteins are the main mechanism by which Hh proteins are transported and whether lipoprotein-associated Hh signals through mechanisms distinct from other Hh pools.

SHH can also signal over a long range and regulates the development of many tissues, including the cerebellum. During late mouse gestation, Purkinje neurons produce SHH to promote the expansion of the external granule layer within the cerebellum. Chin Chiang (Vanderbilt U.) demonstrated that Hh signalling also functions earlier in cerebellar development to promote the proliferation of ventricular zone radial glial cells (Huang *et al*, 2009). However, Shh is not expressed in the cerebellum during these early stages. In trying to identify the source of Hh signals at this stage, Chiang discovered that SHH is expressed from embryonic day 12.5 in the choroid plexus, a highly secretory tissue best known for its role in the production of cerebral spinal fluid. Removing SHH from the choroid plexus reduces ventricular zone proliferation, indicating that SHH secreted into the cerebral spinal fluid supports neural progenitor growth.

Other mechanisms by which Hh is distributed were the subject of several fertile discussions. Most Hh-producing cells are epithelial, and Hh proteins can be detected both apically and basolaterally to their epithelial sources. The origin and relative

Fig 1 | Two mechanisms to deliver Hedgehog at a distance. Hh-producing cells (grey borders) use this signal to induce different cell fates in a neighbouring receptive field of cells (blue borders). Hh proteins (orange) are modified by the adduction of cholesterol (red) and palmitate, perhaps in the apical domain of epithelial cells. Hh might be distributed to the receptive field through its association with lipoprotein particles (grey circles), or along basolateral cytonemes. Hh, hedgehog.

importance of the apical and basal Hh populations to patterning were investigated.

Thérond (IBDC, Nice) presented data indicating that Hh can travel both apically and basally to the imaginal disc epithelium, but that it can travel farther apically (Ayers *et al*, 2010). A mutant form of Patched (Ptc) that is not internalized was expressed in clones to act as a trap and to reveal the amount of Hh that the clones encounter. Clones close to the anterior–posterior border show extensive accumulation of Hh both apically and basolaterally. By contrast, more anterior clones accumulate apical but not basolateral Hh, suggesting that several cell diameters away from Hh sources there might be more ligand apically than basally. Expression of this mutant form of Ptc in the facing peripodial membrane cells might act as a sink for the luminal Hh pool. Creation of this sink decreased expression of the long-range target gene *Decapentaplegic* (*Dpp*), but not the short-range target gene *engrailed* (*en*), suggesting that apical Hh is important for long-range, but not short-range, signalling.

Guerrero (U. Autónoma de Madrid) observed Hh proteins distributed basolaterally along cytonemes—thin actin-based cellular processes that can extend many cell diameters (Fig 1; Ramirez-Weber & Kornberg, 1999). Guerrero described the processes of Hh apical localization, subsequent internalization and intracellular vesicular transport that directs Hh to the basolateral plasma membrane to allow

loading onto cytonemes. In addition to Hh, Guerrero observed other proteins involved in Hh signalling along cytonemes, including Interference hedgehog (*Ihog*), a Ptc- and Hh-binding protein implicated previously as a Hh co-receptor. In the absence of *Ihog*, less Hh is observed in cytonemes, suggesting that *Ihog* might also function in the transport of Hh into or along cytonemes. One prediction of this model is that removal of *Ihog* from the Hh-producing cells should affect patterning in the anterior wing disc.

Kornberg (U. California, San Francisco) demonstrated that many *Drosophila* tissues, including the wing and eye imaginal discs and the air sac primordia, have cytonemes, and that different cytonemes can have different signalling proteins. Taken with Guerrero's findings, it seems that different cytonemes in a single tissue might have different signalling functions; cells of the posterior wing disc extend Hh-containing cytonemes that run basally to the epithelial sheet, whereas anterior imaginal disc cells extend cytonemes apically to the epithelium that contain the transforming growth factor- β (TGF β) family member *Dpp*. These signalling events might feed back on the orientation or stabilization of these cytonemes; Kornberg showed that cytonemes displaying green fluorescent protein (GFP)-tagged TGF β receptor can orient to ectopic sources of *Dpp*, but not to ectopic Hh or Epidermal growth factor (*Egf*). By contrast, ectopic Fibroblast growth factor (*Fgf*), but not Hh, EGF or *Dpp*, can misorient Fibroblast growth

factor receptor (Fgfr)-containing cytonemes in the air sac primordium. Indeed, different air sac primordial cytonemes express tagged versions of TGF β receptor and Fgfr. Together, these results suggest that cytonemes can be dedicated to different signalling pathways, can present a signal or respond to a signal, and can run apically or basally to an epithelium. It will be exciting to discriminate between these functions to determine how much *Dpp*, *Fgf*, Hh and *Egf* signalling depends on cytonemes.

Henk Roelink (U. California, Berkeley) presented work indicating that mechanisms of Hh release from producing cells might also function in receiving cells. Dispatched (*Disp*) is a multipass transmembrane protein recognized previously for its role in Hh-producing cells, in which it is required for Hh release (Burke *et al*, 1999). Consistent with this function, Roelink demonstrated that expression of a defective form of *Disp* causes the accumulation of SHH in producing cells *in vitro* (Etheridge *et al*, 2010). A form of SHH that is not modified by cholesterol is not retained by this mutant *Disp*, supporting the view that *Disp* acts specifically on cholesterol-conjugated Hh (Burke *et al*, 1999). Cells expressing SHH can repress Pax7 expression in co-cultured embryonic stem cell (ESC)-derived neural progenitors, similar to the function of SHH in the neural tube. Replacing wild-type ESCs with those lacking *Disp* resulted in decreased Pax7 repression, indicating that *Disp* functions in the responding field

of cells (Etheridge *et al*, 2010). Determining whether Disp is acting in the responding field to distribute the SHH produced by the neighbouring cells, in the release of low-level SHH produced by cells within the patterning field itself, or by a third mechanism, will provide further important insights into mechanisms of Hh interpretation.

Other proteins with several roles in Hh signalling include the Glypicans. Glypicans are glycosylphosphatidylinositol (GPI)-linked heparan sulphate proteoglycans implicated in multiple signalling pathways including Wnt, Fgf and Hh. *Drosophila* have two Glypicans, Dally and Dally-like (Dlp), with both unique and overlapping roles in Hh signalling. Dally is involved in regulating the range of Hh activity, as Théron demonstrated that loss of Dally in posterior wing imaginal disc clones reduces the range of Dpp expression; a phenotype opposite to that caused by the expression of a secreted, non-GPI-linked version of Dally (Ayers *et al*, 2010). Notum is a lipase that can cleave the GPI linker from Dlp, causing its release from the plasma membrane. Théron showed that loss of Notum function counteracts the effects of Dally overexpression, suggesting that Notum cleavage of Dally also modulates Hh signalling (Ayers *et al*, 2010). Raising the possibility that Glypicans can function in Hh-producing cells, lipoprotein particles, cytonemes and receiving cells, Eaton observed that Dally was found in the Hh-containing lipoprotein particles; Guerrero showed that the other *Drosophila* Glypican, Dally-like, associates with Disp and is found in exovesicles and cytonemes; and Satyajit Mayor (National Centre for Biological Sciences, Bangalore) demonstrated that Dally-like was found in puncta on the apical surface of peripodial membrane cells in association with a GFP-tagged version of Hh. Mayor also found evidence that Hh forms an oligomeric form that associates with Glypicans.

Part of how those receiving cells transduce the Hh signal might involve regulating the acetylation of the downstream transcription factor Cubitus interruptus (Ci). Building on his whole genome RNA interference screen in flies, and previous work from Gulino (Sapienza U.) indicating that vertebrate homologues of Ci can be potentiated by deacetylation, Kent Nybakken (Boston Biomedical Research Institute) examined the role of the histone deacetylase HDAC3 in fly Hh patterning (Canettieri *et al*, 2010; Nybakken *et al*, 2005). Similarly

to vertebrate GLI1 and GLI2, *Drosophila* Ci can be acetylated. Nybakken showed that inhibiting HDAC3 decreased Hh pathway activity in wing discs. HDAC3 is best known for its role in chromatin modification, but Nybakken detected most HDAC3 in the cytoplasm, where it could function in the pathway to mediate activation of Ci.

The role of Hh in various stem cells of the skin was a hot topic. Alexandra Joyner and colleague Isaac Brownell (Sloan-Kettering Institute) used genetic fate mapping of progeny of GLI1-expressing hair follicle bulge cells to identify a Hh-responsive stem cell population that contributes to all cells in the hair follicle, but not the interfollicular epidermis. Interestingly, these cells contribute to the interfollicular epidermis after wounding. Toftgård (Karolinska Institute) expanded on his recently published work that used LGR5 and LGR6 as markers of stem cells in the skin, proposing that the LGR5-expressing cells in the bulge might be a cell of origin of basal cell carcinoma (BCC; Jaks *et al*, 2008; Snippert *et al*, 2010). On wounding, progeny of LGR5-expressing cells can also move into the interfollicular epidermis and sebaceous glands to promote wound repair. The wound environment also seems to reprogramme the developmental capacity of some hair follicle cells and accelerate the development of BCC.

Having covered manifold aspects of Hh biology, the last session focused on the development of anti-cancer drugs. Scientists from no less than four companies presented Hh pathway inhibitors (HPIs) currently in clinical trials. Fred de Sauvage (Genentech) presented data regarding the treatment of metastatic and locally advanced BCC with GDC-0449 (Rudin *et al*, 2009; von Hoff *et al*, 2009), as well as the design of ongoing phase II trials. In addition to the published SMO mutation, novel mechanisms of resistance to HPI in mouse *Ptc^{+/-}p53^{-/-}* medulloblastoma tumours involving amplification of GLI2 and cyclin D1, Hh pathway components downstream from Smo were described. Silvia Buonamici (Novartis) observed a high incidence of resistance in similar medulloblastoma models treated continuously with LDE225 and other HPI. Interestingly, the emergence of tumours resistant to HPI could be delayed by combination of LDE225 with a phosphatidylinositol-3 kinase inhibitor. Karen McGovern (Infinity) and Kelly Bennett (Bristol-Myers Squibb) presented data on IPI-926 and BMS-833923, respectively—two SMO antagonists in phase I clinical

trials. IPI-926 is effective in mutation-driven models, as well as in models involving paracrine Hh signalling, in which the tumour cells produce Hh ligand that activates the pathway in tumour stroma. An effect on tumour-initiating cells was also proposed for IPI-926 in small-cell lung cancer. Preclinical activity of BMS-833923 in various models was described, as well as rationale for targeting tumour-initiating cells in multiple myeloma. Details of the multiple myeloma clinical trial, with an emphasis on addressing the cancer stem cell hypothesis, were presented.

Given the excitement that exploring Hh function continues to provide, the spirit of the meeting was guided less by Baroness Beatrice than by another member of the French Ephrussi. Boris Ephrussi bolstered the modern synthesis of genetics and embryology by deepening our understanding that genes regulate both the intracellular and extracellular control of development. This meeting, covering everything from fly imaginal disc biology to novel cancer therapies, is testament to the power of that synthesis. The next Hh meeting, to be held in two years in Singapore, will undoubtedly teach us more about how Hh proteins are received and interpreted, how the cilium transduces the Hh signal in vertebrates, how Gli transcription factors are activated, whether HPIs are effective cancer therapies, and much more.

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Jeremy F. Reiter is at the Department of Biochemistry and Biophysics, Cardiovascular Research Institute, University of California, San Francisco, CA, USA.

E-mail: jeremy.reiter@ucsf.edu

Frederic J. de Sauvage is at the Department of Molecular Biology, Genentech, South San Francisco, CA, USA.

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