A feast for the senses: development and function of sensory systems

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The EMBO Workshop 'Frontiers in Sensory Development' took place in May 2011 in Barcelona. The meeting brought together a diverse group of scientists to tackle the formation and function of the sensory nervous system in all its complexity. The discussions ranged from how signalling and transcriptional networks control cell identity, architecture and behaviour, to how connectivity is established and how such networks have evolved to generate functional diversity.

Barcelona was an appropriate location in which to host the second EMBO 'Frontiers in Sensory Development' workshop, with sensory stimulation always just around the corner. In a city spiced with Gaudi's distinctive architecture, Miro's art, beautiful vistas of the Mediterranean Sea, tempting wine and food, and the melodious Catalan language on the streets, Berta Alsina (U. Pompeu Fabra, Spain) and Andy Groves (Baylor College of Medicine, USA) attracted around 90 scientists to tackle the sensory nervous system. Rather than focusing on individual senses, the meeting brought together visual, olfactory and mechanosensory systems, and the mix of invertebrate and vertebrate models provided an excellent background for discussion.

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The challenging question that several researchers are addressing is how do complex tissue interactions combine to build sophisticated sensory organs? Surprisingly, such complexity is achieved with just a few signalling pathways that are used repeatedly. A good example of this principle is signalling during inner-ear development. The inner ear forms from a simple primordium, the otic vesicle. Localized sensory patches then generate both neurons and sensory hair cells that are arranged into stereotyped patterns

during morphogenesis (Ohyama *et al*, 2007). Tanya Whitfield (U. Sheffield, UK), Christina Pujades (U. Pompeu Fabra) and Doris Wu (National Institute on Deafness and Other Communication Disorders, USA) each focused on the role of the hedgehog-family protein sonic hedgehog (Shh) in ear formation. From Tanya Whitfield's and Christina Pujades's work, it has become clear that Shh patterns the otic vesicle by establishing its axes, and Doris Wu reported that, at later stages, a local Shh source provides temporal control of hair-cell differentiation along the cochlea, as revealed by conditional inactivation of Shh in mouse. At this stage, other signalling pathways are

also involved, such that intermediate levels of bone morphogenetic protein (BMP) signalling are required for the specification of the sensory domain, as reported by Taka Ohyama (House Ear Institute, USA).

Reiterated use of signalling is also found during the development of other sensory systems. In the case of the olfactory system, Lena Gunhaga (Umeå Centre for Molecular Medicine, Sweden) described the way in which, at first, BMPs inhibit specification of the sensory epithelium and promote respiratory fate (Maier *et al*, 2010); later, BMPs seem to promote olfactory neurogenesis. Several roles for BMPs were also described for retinal development by Yas Furuta (MD Anderson Cancer Center, USA), who suggested that distinct signalling thresholds are needed for dorsoventral patterning, retinal ganglion cell outgrowth and photoreceptor specificity. Linda Barlow (U. Colorado School of Medicine, USA) reported that canonical Wnt signalling is crucial for taste-bud development, as well as for taste-bud regeneration in adults. Wnt first specifies bud progenitors and then promotes receptor-cell differentiation.

Changes in the allocation of signals might also lead to evolutionary diversity. BMP signalling was implicated in the development of the mucus-secreting casquette (the French for 'cap') found in the teleost *Astyanax*. Sylvie Retaux (CNRS Institut de Neurobiologie Alfred Fessard, France) hypothesized that the casquette is homologous to the cement gland of amphibians, albeit with a different position and origin. Similarly to the *Xenopus* cement gland, the

casquette expresses *dlx*, *pitx* and *BMP* genes and is innervated by the trigeminal ganglion. These results suggest that evolutionary recruitment of BMP signalling pathways might allow homologous attachment organs to differentiate in different positions.

Thus, the sequence, duration, level and combination of signals are crucial for normal patterning, cell identity and differentiation. How are different inputs integrated? The lateral line in zebrafish provides an excellent model in which to address this question. Induced in the head, its primordium migrates along the body axis and deposits neuromasts in its wake. Tatjana Piotrowski (U. Utah, USA) summarized the way that canonical Wnt and fibroblast growth factor (FGF) signalling control this process, with Wnt dominating at the migrating front and FGF at the trailing edge. Ajay Chitnis (National Institute of Child Health and Human Development, USA) presented a mathematical model to account for the spatial switch between the two signalling domains, on the basis of Turing's reaction-diffusion system. A combination of local inhibition and long-range activation sets up a progression from a Wnt-dominated zone to one in which FGF has the upper hand. When combined with regulated local proliferation at the primordium front, the signalling cross-talk provides a steady production of neuromasts from the trailing edge. Chitnis's model reveals that a simple genetic network has remarkable potential for complex patterning. The next challenge will be to uncover the intracellular pathways that integrate and compartmentalize these signals.

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The interpretation of incoming signals mostly depends on the developmental history of the receiving cell and its genetic make-up. The combinatory action of transcription factors is at the centre of developmental-cell-fate decisions, and the development of sensory systems is no exception. Several speakers explored the way in which transcription factors regulate different phases of sensory differentiation. In the inner ear, sensory precursors are specified by pro-neural factors in a

common pro-sensory domain; neuronal precursors require *Neurog1*, whereas hair-cell precursors depend on *Atoh1*. Joana Neves (U. Pompeu Fabra) described the way in which the transcription factor *Sox2* interacts with Notch signalling to maintain prosensory precursors and promotes their differentiation through activation of *Atoh1* and *Neurog1* (Neves *et al*, 2011). *Sox2* directly activates *Atoh1*, but its down-regulation is needed for *Atoh1* expression to occur. Regulation of *Sox2* itself must therefore be crucial for the transition from progenitor to differentiation. Although several studies emphasize common regulation of sensory neuron and hair-cell precursors in the otic vesicle, Stefan Hans (Technical U. Dresden, Germany) presented evidence for distinct transcriptional networks. Neuronal precursors require *Foxi1*, whereas hair cells require *Dlx* transcription factors. Understanding the way in which common and distinct regulatory networks interact for proper differentiation to occur is a challenge for the future.

Ellen Lumpkin (Columbia U., New York, USA) provided evidence for a different role for *Atoh1* in sensory development, as a key regulator of Merkel cells (Maricich *et al*, 2009). Merkel cells, which are innervated by peripheral sensory neurons, are found in areas of high tactile acuity within touch domes in the skin. Conditional inactivation of *Atoh1* eliminates Merkel cells without blocking touch-dome formation, and results in the loss of a class of slow-adapting light-touch response. Through a combination of lineage markers and Cre driver lines, Lumpkin and colleagues have demonstrated that Merkel cells are derived from bipotent epidermal progenitors. These progenitors are necessary for Merkel cell renewal and could also be the source of Merkel cell carcinoma.

A deeper understanding of gene regulatory networks might provide insight into their evolutionary origins. Study of the marine annelid *Platynereis dumerili* has revealed mechanisms for the development of sensory structures in the Lophotrochozoa, adding this taxon to models of homology (Arendt *et al*, 2009). Detlev Arendt (European Molecular Biology Laboratory, Heidelberg, Germany) and colleagues analysed gene expression by using image registration to develop profiles of transcription-factor expression in the central nervous system. Maria Tosches (European Molecular Biology Laboratory) described a set of ciliary photoreceptors with pineal-like functions that

share gene expression with vertebrates. These studies suggest that the specification of photoreceptor, chemosensory and neurosecretory cell types have common regulatory mechanisms across taxa.

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The reiterated use and modification of regulatory networks might also provide mechanisms to develop tissue complexity. In the fly, the retinal determination network controls photoreceptor specification in the developing compound eye (Kumar, 2010). Fernando Casares (Centro Andaluz de Biología del Desarrollo, Spain) reported that the same transcription factor interactions also specify their targets in the lamina of the optic lobe. Similarly to in the eye imaginal disc, the homeobox factor *homothorax* (*hth*) is required to maintain proliferating progenitors. It does so by repressing the differentiation factors *eyes absent* (*eya*) and *sine oculis* (*so*), and in turn *eya* represses *hth*. Thus, mutual repression between both factors regulates the balance between proliferation and differentiation. On the basis of the similarities in their transcriptional control, Casares and colleagues proposed that, over the course of evolution, photoreceptors and laminar neurons arose from a common progenitor. Importantly, distinct enhancers control the expression of the *eya/so* target *dac* in both cell types, suggesting that changes in *cis*-regulatory DNA might be the driving force for cell 'speciation'.

Claude Desplan (New York U., USA) focused on the differentiation of neuronal subtypes within the medulla of the optic lobe. Genetic-tracing techniques reveal a columnar organization of retinotopic input, local interneurons and projection neurons to higher brain centres. Additional non-columnar neurons add to the complexity. Medullary neurons come in several recognizable 'flavours', and could comprise more than 60 types of neuron. As for the retina, neurons differentiate in a temporal wave and respond to a distinct wave of retinal innervation. Some neuroblasts generate distinct neuronal types in a sequential manner that is reminiscent of embryonic neuroblasts, and they similarly depend on a scheduled cascade of specific transcription factors. Thus, although development of the

optic lobe shares aspects with the retina, its differentiation is more complex.

Neuronal specification requires pro-neural transcription factors. How do these factors feed into differentiation genes to establish neuronal diversity and function? Andrew Jarman (U. Edinburgh, UK) tackled this question by comparing neurons in *Drosophila* chordotonal and external sense organs. Surprisingly, ciliogenesis genes required for sensory dendrite formation are present in chordotonal neurons long before they acquire their characteristic morphology (Cachero *et al*, 2011). The pro-neural factor Atonal is key for their activation; some ciliary genes are direct Atonal targets, whereas others are activated through intermediate transcription factors. Among these are Rfx, a known regulator of core ciliogenesis genes, and the forkhead transcription factor Fd3f, which seems to regulate subtype-specific genes. Thus, a model emerges in which factors involved in specification of the neuronal lineage initiate a subtype-specific programme at the same time.

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We know little about the link between ciliogenesis and neuronal function. Further clues are likely to emerge from studies in *Caenorhabditis elegans*. With the ability to manipulate function and to visualize individual cilia of defined sensory neurons *in vivo*, the worm offers the unique opportunity to investigate the genetic mechanisms required to generate and modulate ciliary structures. By using this strategy, Piali Sengupta (Brandeis U., USA) reported the identification of a new transmembrane protein that interacts with known regulators to control transport within cilia. Indeed, its mutation causes not only a branching phenotype in sensory neurons, but also defects in chemosensory perception. Thus, neuronal morphology is clearly linked to function.

In the vertebrate inner ear, ciliary geometry is more complex: on each hair cell, stereocilia are arranged in a V-shape of decreasing length, with a kinocilium at the vertex. The kinocilium is crucial to patterning of the entire bundle. Raj Ladher (RIKEN Center for Developmental Biology, Japan) presented evidence that FGF signalling is

required for kinocilium formation and for stereocilia arrangement. In addition to their intrinsic apical–basal polarity, hair cells are aligned with each other along the longitudinal axis of the cochlea. Non-canonical Wnt PCP, signalling is thought to control both aspects of polarity (Chacon-Heszele & Chen, 2009). However, careful analysis in mutant mice suggests otherwise. Ping Chen (Emory U., USA) showed that, in the absence of kinocilia, stereocilia are disorganized, but the distribution of PCP components remains normal. By contrast, when PCP-pathway components are lost, their characteristic subcellular localization is disturbed and hair cells are misaligned, but their intrinsic apical–basal polarity remains intact. Thus, hair-cell polarity can be uncoupled from their arrangement along the cochlea. How does PCP activity translate into elongation of the sensory epithelium? It seems that the classic adhesion molecule E-cadherin is one of the mediators downstream from PCP signalling. The next challenge will be to determine the way in which FGF, PCP and cilia formation are integrated on a cellular level to coordinate cochlear morphogenesis and cilia formation.

In parallel to cell specification and morphogenesis, sensory circuits must be organized into topographic maps to represent the visual, auditory and olfactory world in the central nervous system. Different sensory systems and species use distinct strategies to achieve this. In the fly visual system, retinotopic connections are complicated because of the unique geometry of the eye. Neighbouring photoreceptors in the same ommatidium sample different visual spaces, and photoreceptors in the same position in a different ommatidium sample the same spot. The latter project to the same central targets, whereas axons from the same ommatidium project to different targets. Tom Clandinin (Stanford School of Medicine, USA) showed that the atypical cadherin Flamingo has a crucial role in photoreceptor axon-target choice (Chen & Clandinin, 2008). Surprisingly, Flamingo acts non-cell-autonomously. Individual growth cones seem to compare the relative levels of Flamingo activity in their neighbours, which they interpret as attractive or repulsive cues. When individual cells lose Flamingo, their neighbours detect an imbalance in adhesive forces, ultimately resulting in mistargeting. Thus, growth cones seem to measure local changes in cell–cell adhesion and translate this into differences of target selection.

The olfactory system of vertebrates has a different kind of spatial map, in which individual glomeruli of the olfactory bulb receive projections from cells with wide-spread, random distribution throughout the olfactory epithelium, but all express the same olfactory receptor. However, as for the fly visual system, local interactions seem to be important for formation of the sensory map. Takeshi Imai (RIKEN Center for Developmental Biology) demonstrated that coarse mapping occurs with sorting of axons as they project to the olfactory bulb. Levels of cyclic AMP regulate differential expression of Neuropilin receptor and Semaphorin ligand, allowing axons to sort according to the relative levels of the receptor–ligand pair (Imai *et al*, 2009). Although axon sorting requires olfactory receptors, it occurs in the absence of odorants, suggesting that intrinsic activities of receptors might determine axon targeting.

As was apparent from the diverse presentations in Barcelona, our understanding of the development of sensory systems is enjoying a period of rapid progress. The field has moved from classic developmental and evolutionary biology to incorporate stem-cell biology, mathematical modelling, genomic and systems approaches. The complexity of sense organs and sensory circuits promises challenging and exciting times ahead to understand the molecular control of cell-fate decisions, patterning, connectivity and the evolution of form and function.

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