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Wiring the nervous system: from form to function

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The RIKEN Centre for Developmental Biology recently hosted a joint UK-Asian Pacific Developmental Biology Network meeting called 'Development and Emergence of Function in the Nervous System'. The meeting's program, which was organized by James Briscoe and Krishnaswamy VijayRaghavan, covered a spectrum of processes and mechanisms in neurodevelopment, ranging from the patterning of neural tissue to the initiation of a functional nervous system. One idea to have emerged during this meeting is that 'form underlies function'. Here we discuss some of the themes that were addressed and provide a broad impression of what was a highly stimulating and successful conference.

Introduction

The nervous system is an organ of enormous complexity, and its research brings together diverse fields, such as genetics, molecular and cell biology, physiology and behavioral biology. In the past, studies of neural development mainly followed a molecular morphogenetic approach, whereas those involving the post-embryonic nervous system relied more heavily on electrophysiology and functional output. Contemporary developmental neurobiology is now breaking down the barriers that once existed between these two formerly more distinct disciplines of research. This meeting aimed to provide an overview of the 'development and emergence of function in the nervous system' within the context of this current trend in neurodevelopment, and brought together researchers working on a variety of model organisms, ranging from *Drosophila* to mice.

Pattern formation in the nervous system

The assembly of functional neuronal circuits depends on the correct specification of the various cell types in the developing nervous system. This was exemplified by work describing the specification of neuronal subtypes in the vertebrate neural tube and the function of graded Hedgehog (Hh) signaling in this process. James Briscoe (National Institute of Medical Research, London, UK) showed that 2- to 3-fold changes in Gli activity in the chick spinal cord mimic the switch in neuronal subtypes seen upon 2- to 3-fold changes in Hh concentrations. He found that neural progenitors can integrate both the duration and concentration of sonic hedgehog (Shh) levels to determine their neuronal identities, and that this is mediated by a progressive desensitization to Shh signaling (Stamataki et al., 2005). The diversity of motoneuron subtypes is also known to be specified by unique combinations of the LIM-homeodomain transcription factors, which are thought to regulate the expression of downstream guidance receptors and ligands. Ryuichi Shirasaki (Osaka University, Osaka, Japan) showed that the mouse dermomyotome is a source of a secreted long-range attractant that is specific for a motoneuron subtype. Interestingly, the fibroblast growth factor receptor 1 (FGFR1) is specifically expressed in this motoneuron subtype, which is genetically defined by the LIM code during axon pathfinding. Furthermore, the LIM code programs these motoneurons to express FGFR1 in vivo, providing insights into how downstream effectors of the LIM code direct wiring in the developing central nervous system (CNS) (Shirasaki et al., 2006). The specification and patterning of interneurons in the zebrafish neural tube was described by Kate Lewis (University of Cambridge, UK). Unlike motoneurons, the axons of interneurons remain within the CNS and their development is not as well understood. Lewis described her laboratory's studies into the role of Hh, retinoic acid and Notch/Delta signaling in the formation of zebrafish CiA interneurons (which are analogous to V1 cells in amniotes).

Evolution of placodal complexity

Although the development of the CNS of all bilateria shares some commonalities, many additional cell types have evolved independently within each group. The underlying molecular basis for this complexity can be revealed by comparative analyses of the cell types and the signaling pathways that govern their diversification. Sebastian Shimeld (University of Oxford, UK) discussed the development of sense organs and, in particular, of placode-like cell types in the sea squirt *Ciona intestinalis*. Although this simple chordate does not have a recognizable lens placode, many of the genes that regulate the development of the lens placode (for a review, see Medina-Martinez and Jamrich, 2007) in higher vertebrates are present in the *Ciona* genome; these genes are expressed in the anterior and lateral invaginations that later form the oral siphon, the neural complex or the atrial siphon, structures that are all associated with 'sensory' functions (Shimeld et al., 2005). Tanya Whitfield (University of Sheffield, UK) discussed the development of the otic placode in zebrafish, and compared it with that of a jawless fish, the lamprey. Whereas Hh signaling is necessary and sufficient for posterior identities in the developing zebrafish ear, changes in *otx1* expression (rather than changes in Hh signaling) can account for all the major differences between the inner ears of jawed and jawless embryos. In particular, the acquisition of a domain of *otx1* expression appears to have generated the differences in ear development between agnathans and gnathostomes (Hammond and Whitfield, 2006).

Neurogenesis

Neurons derive from neural progenitors within the neuroepithelium. Mitotic progenitors generate one daughter that is a functional self-copy and a second that becomes a postmitotic neuron, a process that in many species is achieved by asymmetric cell division (Yu et al., 2006). The neuronal progenitor neuroblast cells of *Drosophila* provide a model system that is widely used to study this form of cell division, and two talks highlighted aspects of the regulation of self-renewal and differentiation in these cells and their progeny. On dividing, a neuroblast generates a small, more differentiated daughter known as a ganglion mother cell, which inherits the homeodomain protein Prospero. Andrea Brand (Gurdon Institute, University of Cambridge, UK) described how a genome-wide survey using the dam methylase modification method revealed that Prospero binds to and represses the transcription of a number of

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genes necessary for neuroblast function, such as those involved in cell cycle regulation. Interestingly, Prospero also simultaneously binds to and activates genes involved in neuronal differentiation, suggesting that this protein serves as an important gene expression pattern switch that operates from proliferation through to terminal differentiation. She also described an elegant method used to show that ganglion mother cells undergo multiple cell divisions in the absence of Prospero, reconfirming Prospero's role at the cell biological level (Choksi et al., 2006).

Neuronal polarity and growth cone guidance

Neurons extend multiple processes known as neurites, one of which is specified as the axon, whereas the remainder become dendrites. Although the establishment of this neuronal polarity requires extensive remodeling of microtubules and the actin cytoskeleton, the transport of membrane components to the growth cone is also essential in axon extension. Michiko Shirane (Kyushu University, Fukuoka, Japan) showed that protrudin, a MAP-K-regulated factor, participates in this process by binding to the GTPase RAB11, resulting in the positive regulation of membrane recycling during axonal extension (Shirane and Nakayama, 2006).

The mechanisms that determine from which points on the surface of the neuronal cell body neurites begin to extend and how one neurite is selected to be the neuron's sole axon remain obscure. In recent years, the role of the centrosome has come into focus as a site that may integrate intrinsic and extrinsic cues to bias the selection of one neurite. Guy Tear (King's College London, UK) suggested that Mushroom body defect (Mud), a *Drosophila* homolog of NuMA, might be involved in this process. He showed that this protein localizes to the neuronal centrosome and that a lack of Mud function can result in defects in axon outgrowth.

Mu-ming Poo (University of California, Berkeley, USA and Shanghai Institutes for Biological Sciences, Shanghai, China) is known for his series of demonstrations that the turning response of growth cones toward a chemoattractant is determined by differences in the concentrations of cAMP and cGMP, and that the true intracellular mediator of this mechanism is local changes in the concentration of Ca^{2+} ions (Henley and Poo, 2004). In his talk, he extended the analysis to axon-dendrite selection. In his model, external signals that induce axonogenesis trigger downstream positive-feedback cascades, allowing one neurite to be committed to an axonal fate, while the others passively undergo dendritic differentiation. This scenario might open up new perspectives into the establishment of neuronal polarity.

Projection

The global patterning of axonal projections is of fundamental interest. In the visual system, continuous positional information in the retina determines the projection destinations of individual neurons, and some form of fine-tuning appears to be required in order to maintain nearest-neighbor interactions between neurons. In *Drosophila*, retinal axons release two anterograde signals – Hh and Epidermal growth factor (Egf) – on entering the lamina, prompting the formation and differentiation of target neurons. In her talk, Iris Salecker (National Institute of Medical Research, London, UK) proposed that the ligand Jelly belly and Anaplastic lymphoma kinase work together to form a third anterograde pathway that mediates the fine-tuning of retinal axon targeting (Bazigou et al., 2007).

The olfactory sensory system exhibits a different set of properties. In the fly, about 50 olfactory sensory neuron subtypes [defined by their exclusive expression of a single, specific odorant receptor (OR)] project to the primary olfactory center, known as the antennal lobe. This is in contrast to the mouse, which has 1000 types of OR. The expression of ORs in the mouse is highly specific, with a single gene encoding a specific receptor (the 'one neuron – one gene' principle). Axons from neurons that express the same OR converge on the same glomeruli (relay units in the antennal lobe), thereby transforming olfactory sensory input into a discrete positional code. Chihiro Hama (RIKEN Centre for Developmental Biology, Kobe, Japan) presented evidence that in *Drosophila*, the on-off state of the Notch signaling pathway plays a part in this process (Fig. 1A). Olfactory sensory neurons are derived from sensory organ precursors (SOPs) in the antennal anlage via asymmetric cell divisions, during which Notch-on classes and one Notch-off class of neurons are generated. The combination of this binary Notch code with the positional information of the precursors within the antennal disc provides a degree of regional guidance to projecting axons and directs the expression of specific ORs, indicating that the Notch on-off combinatorial code is crucial in coupling projection pattern with selective OR expression (Endo et al., 2007).

From the mammalian side of the olfactory fence, Hitoshi Sakano (University of Tokyo, Japan) gave an overview of recent findings from his laboratory (Fig. 1B). In the mouse, projection of olfactory neurons along the dorsal-ventral (DV) axis of the olfactory bulb depends on positional information that relates to the 'birthplace' of neurons in the olfactory epithelium, whereas the anterior-posterior (AP) arrangement of neuronal

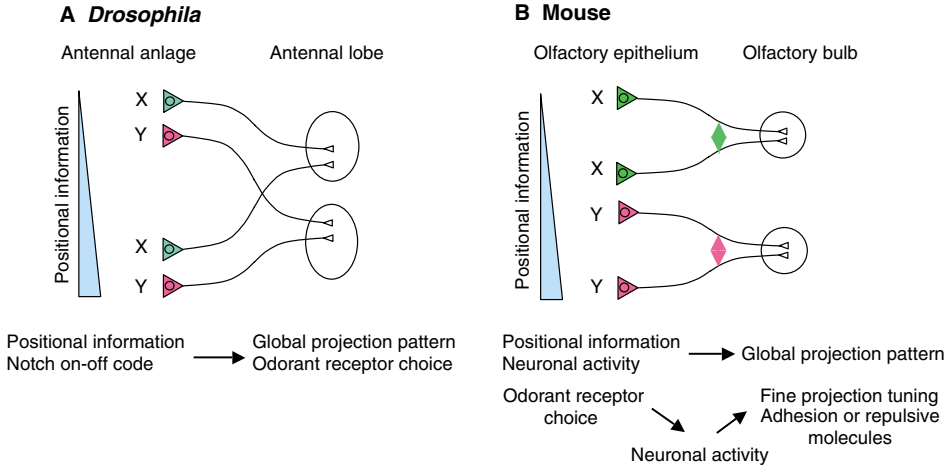

Fig. 1. Olfactory neuron projection in *Drosophila* and mouse. (**A**) In *Drosophila*, the Notch on-off code (red, Notch off; green, Notch on) is involved in coupling antennal neuron axonal projections with their odorant receptor expression. (**B**) In the mouse, the odorant receptor code is transformed into gene expression patterns that regulate axonal fasciculation via neuronal activity (red and green indicate different adhesion molecules). The X and Y labels next to the cell bodies indicate odorant receptor types. The diamonds indicate homophilic adhesions.

projection depends less on epithelial location and more on the strength of cAMP-PKA (PRKACA – Mouse Genome Informatics) signaling downstream of the OR. The choice of OR is restricted, to some extent, by the positional information that olfactory neurons possess. The decision of which glomerulus the termini project to depends on the OR that is expressed. Running counter to conventional models, Sakano provided compelling evidence for a scheme in which ORs guide axon projection, but indirectly in an activity-dependent manner. He showed that the expression levels of the adhesion molecules Kirrel2 and Kirrel3 are modulated by the specific OR expressed by a given neuron; if one is high, the other is low. The ratio of Kirrel2 to Kirrel3 is determined in a cAMP-gated channel-mediated fashion by neuronal activity in response to the activation of ORs. The repulsive guidance molecules EphA5 and ephrin A5 display similar behavior. Elegant molecular mosaic experiments showed how the targeting of axons expressing the same ORs to the same glomeruli can be explained by the control of the fasciculation and segregation of axons by these pairs of genes (Serizawa et al., 2006). Sakano's model of the preliminary roadmaps that are laid down by positional cues and modulated by neuronal activity-dependent fine-tuning, indicates that the general principles of neurogenesis hold true in the mammalian olfactory system as well.

In other talks, Paul Whitington (University of Melbourne, Australia) spoke about several guidance molecules that function at specific switch points in pathfinding *Drosophila* sensory neurons, and Alain Ghysen (INSERM, Montpellier, France) introduced novel and fundamental work on how lateral line neurons project to the CNS in zebrafish in a manner he described as "touching at a distance".

Formation of functional circuits

Repetitive stereotyped behavioral patterns, such as walking and respiration, arise from the programmed firing of motoneurons that are organized into neural circuits. It is thought that these neural networks, known as central pattern generators, are genetically programmed, but how the functional circuit is formed remains obscure. Michael Bate and Sarah Crisp (University of Cambridge, UK) have taken the neuronal activity that drives the sequential muscle contractions along the AP axis of the *Drosophila* larval body, which underlie the wave-like crawling movements of larvae, as a model for studying the development of a motor circuitry. By visualizing muscle activity using GFP technology, they made real-time observations of the initiation of muscular contractions in late-stage embryos and found that the development of motor activity patterns over time is highly reproducible. This raises the possibility that the central pattern generator engages in the self-tuning of its circuitry via the spontaneous activity of its constituent neurons as it develops.

As the fruit fly metamorphoses from a crawling larva into a walking, flying adult, its nervous system undergoes massive rearrangements. Darren Williams (MRC Centre for Developmental Neurobiology, King's College London, UK) introduced his work on neuron remodeling and the non-apoptotic role that caspase proteases play in large-scale pruning. Alongside this, he described the global role that lineage-specific apoptosis plays in generating adult-specific circuits and how it reveals the modular way in which the nervous system is generated from developmental units termed 'hemilineages'. Krishnaswamy VijayRaghavan (National Center for Biological Sciences, Bangalore, India) reported that, upon metamorphosis, several re-arborization events involving motoneurons and interneurons depend on the moulting hormone ecdysone and the intracellular cascade downstream of canonical Wnt signaling. A genetic approach revealed that the post-eclosion changes in these neurons depend on activity, once again highlighting the importance of activity-dependent fine-tuning in neural circuit formation.

In the mammalian hippocampus and olfactory nervous system, new neurons are continuously born even into adulthood, and it is believed that apoptotic cell death serves as a mechanism for pruning away incorrect or unnecessary neurons. Woong Sun (Korea University College of Medicine, Seoul, South Korea) discussed a knockout mouse engineered to lack the pro-apoptotic gene *Bax*, the study of which revealed that apoptosis plays an adaptive role in fundamental brain functions, such as associated learning.

Plasticity

There is growing evidence that the molecules that control neural circuitry development also represent the building blocks of experience-driven plasticity in the adult brain. Dendritic growth, spinogenesis, facilitation of synaptic plasticity mechanisms and the enhanced expression of neurotrophins, are processes that are involved in experience-driven plasticity that also occur during hippocampal and cortical development. Shona Chattarji (NCBS, Bangalore, India) showed that many of the plasticity mechanisms above are also triggered in the adult amygdala, the emotional hub of the brain, following an aversive experience. He found that in rats exposed to chronic stress, dendritic arbors and spine density are increased in the projection neurons of the lateral amygdale (Vyas et al., 2003). A positive correlation was also observed between spinogenesis and enhanced anxiety-like behavior in transgenic mice overexpressing the neurotrophin BDNF. This structural remodeling is accompanied by electrophysiological changes at excitatory glutamatergic synapses. A rat model of fear-conditioning confirmed that stress experiences cause high levels of fear. Thus, Chattarji suggests that prolonged stress may leave its mark in the amygdala by forming new synapses that have an enhanced capacity for subsequent potentiation, thereby creating an ideal synaptic substrate for the emotional symptoms of stress disorders.

Long-term potentiation (LTP) of synapses is a form of synaptic plasticity that is thought to play a crucial role in behavioral learning and that can be induced by the activation of NMDA-type glutamate receptors. Another form of synaptic plasticity is long-term depression (LTD), one form of which involves the downregulation of AMPA-type glutamate receptors that mainly mediate postsynaptic excitation. LTD can be blocked by LTP. Graham Collingridge (University of Bristol, UK) reported that this interaction is mediated by a kinase cascade. LTP is enabled by the activation of NMDA receptors, which triggers an influx of Ca^{2+} ions and the activation of a pathway that involves the PI3 kinase and protein kinase B (AKT). And, as is seen in signaling downstream of insulin, the end result is the inhibition of glycogen synthase kinase 3 β (GSK3β), which intensifies the downregulation of the AMPA-type receptors that underpin LTD (Peineau et al., 2007). This work shows how controlling the balance of two glutamate receptors can minutely regulate physiological activity at the synapse.

NMDA receptor-mediated LTP begins with local biochemical reactions that occur at and around the synaptic junction, but the maintenance of LTP requires changes in transcriptional regulation as well. Kentaro Abe and Masatoshi Takeichi (RIKEN CDB, Kobe,

Japan) proposed a new mechanism by which postsynaptic signals via the NMDA receptor can be transduced to the nucleus, involving the direct nuclear import of β-catenin, a molecule that also functions as a cytoplasmic binding-partner of the classic cadherins in cell adhesion, and as an important downstream factor in canonical Wnt signaling. Canonical Wnt signal transduction inhibits the degradation of cytoplasmic β-catenin, allowing it to enter the nucleus and regulate the transcription of target genes. Abe and Takeichi found that activation of the NMDA receptor in hippocampal pyramidal neurons causes calpain-mediated cleavage of β-catenin. The resultant molecular fragment resists cytoplasmic degradation and accumulates in the nucleus, where it demonstrably functions as a transcriptional activator for at least one target of the canonical Wnt pathway. In novelty exploration tests, mice showed this same calpain activity-dependent cleavage of β-catenin, evidence that this novel signaling pathway operates in vivo as well (Abe and Takeichi, 2007).

Conclusion

This meeting featured research into nervous system development primarily in *Drosophila*, zebrafish and mouse, but the degree of commonality among the questions that concern the development and functioning of the nervous systems of these phylogenetically distant taxa was nonetheless surprising. One main question addressed was how patterning and projection mechanisms arrange initially undifferentiated cells into a highly orchestrated neuronal circuitry. The relationship between the structural changes that take place in development and the plasticity of functional circuits was also striking. The concept 'form underlies function' emerged from these talks. At the same time, studies described by Sakano, VijayRaghavan and Bate highlighted developmental contexts in which 'function underlies form'. Thus, the relationship between function and form seems more mutually coherent than previously thought. In this sense, the advent of tools and methodologies that simultaneously enable the high-resolution study of the form and function of the neuronal components within a circuit in real time, represents a wonderful opportunity for scientists to push back the horizons and open up new insights as never before.

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