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Epigenetics in cognitive development in Nijmegen, The Netherlands

It's Not All in Your Genes!

Beyond all the wiring that stitches the brain together are the command centres operating from within each neuron, which endow the organ with an exceptional intellectual propensity. Recent research has identified some 'new' performers, the regulators of the epigenome. A rendezvous with Annette Schenck and colleagues tells us more.

Having a bit of a brain fog before crashing into an important meeting can be embarrassing, though is not unusual. With so many dates, deadlines and priority-number-ones crowding our agendas, it's a sure thing that the mind is bogged down. Is it then a paradox that the computational prowess of the brain is exorbitant? That the brain's 100 billion neurons, a figure easily greater than the number of people on earth, make multitasking and parallel-processing easy? Well, not quite. With startling breakthroughs in genetics, neurobiologists have an answer. While genes do their part by establishing neural networks and keeping nerve conduction intact, epigenetic modifications fine-tune neuronal signalling, in effect honing the most intricate aspects of cognition: learning and memory. But at times when the regulators, metaphorically termed 'writers', of the epigenome become dysfunctional, neuronal integrity falls apart and may lead to cognitive handicaps.

Early years

Neuroepigenetics is an emerging trend in life science research, which has been accentuated by groundbreaking contributions describing a role for histone acetylation and DNA methylation in transient and long-term memory and, more recently, in behavioural responses to chronic stress (*Neuropsychopharmacology Reviews,* 36:356-7). The molecular mechanisms that control epigenetic modifications, which are very diverse in different neurons, remain elusive.

Together with her colleague Hans van Bokhoven, Annette Schenck, a molecular neurobiologist and an associate professor at Radboud University Nijmegen Medical Center in The Netherlands, has recently ascribed a role to the histone-methylating protein, euchromatin histone methyltransferase 1 (EHMT1) in cognitive development using *Drosophila* models (*PLoS Biol,* 9(1):e1000569). Their work has opened new doors to the understanding of the 'orchestration' of epigenetic programmes featuring key learning and memory genes.

Even as a doctoral student, Annette Schenck was fascinated by neurobiology and, indeed, chose to address disease-relevant questions in the field. During her stay in Strasbourg, where she worked in the lab of Jean Louis Mandel, Schenck took to the study of Fragile X mental retardation (FMR), a common intellectual disability characterised by transcriptional silencing of the protein FMRP and abnormal synaptic morphology. The project initially set out to identify interacting partners of FMRP. She recollects, "Along with my colleague Barbara Bardoni, I identified a protein, CYFIP, that provided a molecular link to a pathway of other intellectual disability genes and regulators of synaptic morphology. In fact, we stumbled into the Rac1 GTPase pathway. This was so exciting that I absolutely wanted to know what this link means to the nervous system. We, however, needed an organism that could be quickly genetically manipulated. Angela Giangrande next door, had then been working on the *Drosophila* nervous system. By characterising the fly proteins that caused the phenotype and with a combination of biochemical and genetic experiments, we were able to dissect a novel pathway that controls neuronal connectivity."(*Neuron,* 38(6):887-98).

Annette Schenck (second from right) and the rest of the neuroepigenetics group is flying high in *Drosophila* research

Following her PhD, Schenck felt the need to venture into something new and unknown. Her interests brought her to the MPI-CBG in Dresden and she turned back to basic cell biology. Together with her mentor, Marino Zerial, she investigated membrane trafficking in a developmental context, focusing on the function of a novel endocytic organelle in the zebrafish (*Cell,* 133(3):486-97). As a group leader today, she works on elucidating molecular mechanisms underlying brain development and applying her research in medicine. She believes that identifying molecular networks of disease genes and their protein partners can help not only to devise diagnostic tools, but in the long run hold valuable therapeutic prospects.

Working with fruit flies…

Annette Schenck does not wish to limit her work to single proteins but aims at getting a, "big picture". The Schenck lab has at its disposal 300 different *Drosophila* models of intellectual disability disorders for extensive and systematic characterisation. Schenck says that the fly is a 'handy' organism, especially when it comes to questions of reverse genetics that require a certain throughput. "Targeting genes of interest is relatively easy and fast in *Drosophila*. One can swiftly perform cell-specific genetic manipulations, since the resources for such work are available in the fly community – be it genome-wide RNAi libraries or collections of transposon insertions. Once we discover an interesting phenotype using these discovery tools, we follow up with generating our own mutants and additional tools," Annette Schenck summarises. Lastly, mounting evidence suggests that several aspects of fly development can be applied to humans, owing to the conservation among developmental genes between the two species.

In their recent paper, the Schenck lab and its collaborators have greatly profited from the use of their fly models. The EHMT-deletion mutants ($EHMT^{DD}$) exhibit stark cognitive defects in learning and memory tasks but perform well in other assays, suggesting the absence of any ambiguity in the

analyses. In addition, the fly has worked well in their adult-rescue experiments. "Genetic rescue in the adult has lately emerged as a common theme in neurodevelopmental disease research. This has truly become an eye-opener, since traditionally we assumed that intellectual disability disorders arise from hard-wiring problems of the nervous system and do not develop from an acute dysfunction. Our results with *Drosophila,* when translated to humans, can imply a potential for successful postnatal treatment of neurodevelopmental disorders," explains Schenck. However, as a word of caution, she does not forget to underscore the limitations of her model system. "One should keep in mind that the fly is indeed far behind in evolution. Though genes of metabolic pathways and signalling are highly conserved, homology in epigenetic genes is only about average," she concludes.

…and reaping the fruits

When Annette started an independent lab in Nijmegen, she had the opportunity to embed her group into a human genetics department renowned for the identification of genetic causes of intellectual disorders. Following Kleefstra's and van Bokhoven's discovery of a clinical syndrome (now known as Kleefstra syndrome, a severe intellectual disability characterised by autistic-like features) and the underlying EHMT gene, Annette Schenck got to collaborate with departmental staff, to whom she refers as "gene hunters". Their EHMT paper came about when they began to look at the morphology of neurons in the EHMT^{DD} flies. They observed a striking decrease in dendritic branching of multi-dendrite sensory neurons in the knockouts. Behavioural studies showed conspicuous defects in larval locomotory patterns, non-associative learning, long-term and short-term courtship memory in these flies. Adult rescue of the memory deficits seen in the EHMT^{DD} flies further demonstrated that EHMT is required for memory in adult flies and that the cognitive defects associated with the loss of EHMT are reversible in the mutant flies. Next, the Schenck lab performed a genome-wide H3K9 (the locus of methylation on histone 3 introduced by EHMT) methylation screen in wild-type and mutant flies and identified several regions on the genome that exhibited a 'loss of methylation', or LOM, upon loss of EHMT. These clusters of LOMs eventually turned out to be located directly at the beginning and at the end of the EHMT target genes. In essence, the screen enabled the identification of a broad variety of genes whose transcription was normally repressed by methylation by EHMT (*PLoS Biol,* 9(1):e1000569).

The EHMT paper shows for the first time the critical role of EHMT in the epigenetic regulation of genes involved in a plethora of functions including specific aspects of neuronal development as well as stress response and actin cytoskeleton remodelling. As she describes her paper, Annette gives a special mention to the fruitful collaboration with Hans van Bokhoven and the work of her post-doc Jamie Kramer, who performed most of the experiments. Jamie recalls his Eureka moment, "It was a great instance when genome-wide profiling of H3K9 methylation in the EHMT^{DD} flies identified targets that make about two-thirds of all known learning and memory genes." He adds, "We will continue studying the molecular networks underlying Kleefstra syndrome concentrating more on the newly identified putative EHMT targets."

The big task

The Schenck lab is a small but highly ambitious group whose primary aim is to apply different aspects of fundamental neuroscience in addressing medically relevant questions. With respect to EHMT, their priority is to identify which of EHMT's putative targets is responsible for its crucial role in cognition and also to address the interplay between EHMT and other components of the protein complex of which it is part. Furthermore, the occurrence of stress-response or plastic genes among their candidates has prompted the Schenck lab to study the effects of mis-methylation in EHMT mutants under specific conditions, including learning, stress and challenging environments. This would derive a role for EHMT in the regulation of transcriptional plasticity besides steady state gene expression.

The Schenck lab is working towards obtaining a comprehensive insight into the pathologies of intellectual disabilities. "We want to understand what it takes for a gene to contribute to intellectual disability. Is it the protein partners, the expression patterns, the cellular (neuronal) localisation or its functions? We intend to look at all intellectual disability genes simultaneously and systematically analyse the data." On a final note, Annette Schenck mentions that a consortium of scientists, GENCODYS, and the European Community have lent immense financial support in their efforts to transfer knowledge from flies to mice and humans.

It looks like the fly lab is all set for a flying start! MADHUVANTHI KANNAN