Imaging of retinal development provides more clues to neural complexities (w/ Video)

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(Medical Xpress)—With an incredible diversity of cell types, the central nervous system (CNS), comprising the brain, spinal cord and retina, can be considered to be the most complex organ in the body. Professor Bill Harris, an experimental biologist and Head of the Department of Physiology, Development and Neuroscience, is fascinated by how this complex and sophisticated system is built out of a collection of undifferentiated cells. By putting an advanced technology to novel use, he has been able to observe for the first time the entire process of retinal development at the cellular level in zebrafish embryos. This has achieved a long-sought goal in developmental neurobiology: a complete analysis of the building of a vertebrate CNS structure in vivo.

Terra incognita

"What surprises me most is the inscrutable logic of it all," said Harris. "Every experiment designed to differentiate between different hypotheses leads down one or other branch of an untravelled winding way, through a complex and cleverly evolved network that eventually leads to the functioning CNS."

"We use the retina as a model for the rest of the brain, and zebrafish are a useful research model species because their transparent embryos are easy to see into, and develop rapidly," he added. The zebrafish process of retinal development is complete by about three and a half days after fertilisation; in humans, it takes up to a year. Focusing on the developing

visual system in this lower vertebrate, Harris has broken the process down into discrete events to unravel some of the mysteries that lead to the formation of a perfectly built nervous system.

"At 24 hours after fertilisation, to an inexperienced eye, all the cells of the embryo look the same," explained Harris. "But already the decisions as to what they will become have been made. The little bulge that is destined to become the eye is now committed, but all the cells within that bulge still have the potential to give rise to various types of retinal neurons. We call those retinal progenitor cells (RPCs)".

Harris' research focuses on understanding how the cellular diversity in the retina arises: how the RPCs produce different cell types, and how, what and when decisions are made. He and his colleagues have found that each RPC seems to behave individually, producing clones of variable size and composition. One of the big puzzles is how, from beginnings that show such variability, a retina of just the right size and cellular composition forms. "The fully formed retina has a hundred different types of neurons. I want to know how it all comes about so perfectly," he said, "it's a terrific puzzle, and a fundamental issue in developmental neuroscience."

Pushing boundaries

Combining imaging with powerful genetic labelling techniques helps to elucidate the fine detail. "We discovered a zebrafish gene that was necessary for the formation of retinal ganglion cells, the first cells to exit the cell cycle and start to differentiate," said Harris. "We used the promoter of that gene to drive a fluorescent protein, then when the gene was turned on just before the final division of the RPC, it made the cell fluorescent," said Harris. These fluorescent cells can then be followed under the microscope throughout retinal development.

Harris uses a sophisticated confocal microscope to take a set of images of labelled cells in the developing zebrafish embryo in a single plane, and then stacks them to build up a 3D view of the cells. By repeating this at regular intervals, a 4D, or time-lapse image, is created over several days that shows the set of undifferentiated cells proliferating and differentiating into particular types of neurons. A pioneer of this approach, he has transformed the way scientists can look at the developing brain. An article due to be published in Neuron this year describes his ground-breaking work.

"Forty years ago you would look at the embryo and label some cells, then later look again to see what cells were now labelled or where they were. You'd get snapshots under the microscope and surmise what happened in between. Now we are actually seeing how the cells divide, which gives us a lot more information to help distinguish certain hypotheses from others," said Harris.

"We've made some spectacular movies where we can actually see the RPCs going through cell division to produce two daughter cells in vivo in the retina. We also see transient processes that people didn't notice before," Harris added. "We see that all the RPCs remain attached to one side of the epithelium by a tiny little thread throughout cell division. Since the 1930s, we've known that the RPCs let go of one side but we'd never seen this thread before. Now we want to know what its purpose is – is it important for keeping a cell arranged in the right way? Does it serve a purpose later on in development?"

Using 4D analysis for hundreds of cells at different stages throughout retinal formation, Dr Jie He, a Postdoctoral Fellow in the Harris lab, found there was a stochastic, or chance, element involved in the types of daughter cells formed each time a cell divides. In collaboration with Ben Simons, Professor of Theoretical Physics at the Cavendish Laboratory, they developed a model to explain this variability in the proliferation of

RPCs, and how, in spite of this variability, RPCs managed to produce a perfect retina every time.

Cambridge Advanced Imaging Centre

Harris is driving the development of a new purpose-built imaging centre – the Cambridge Advanced Imaging Centre (CAIC) – which opens in October 2012 and has been made possible through generous funding from The Wolfson Foundation, the Wellcome Trust and support from the University. By augmenting the best commercial and University-developed instruments, this facility will offer to the broader scientific community the latest developments in light and electron microscopy, adapted to specific scientific questions, with the aim of accelerating progress in research across a broad range of areas.

"CAIC will enable us to look inside a living tissue at any resolution from single molecules to thousands of cells, and see the machinery that's operating there in real time," said Harris. "It took us three years to track the hundreds of cells in our study. Once the new microscopes are built, they will perform at a much higher resolution, and much faster," said Harris. "Our hope is to be able to see all the RPCs dividing and giving rise to all the cells in the retina, and to do this over several embryos and see the variations so that we can properly describe the statistical properties. We also need a lot more data to understand the nature of the stochastic machine that generates the right proportions of different cell types, and CAIC will help us do that."

Human aspect

The process Harris has observed in the zebrafish also occurs in humans, and a challenge for the field will be to understand how the stochastic machine changes so that human-sized retinas come out of a collection of

human RPCs. Beyond the field of neural developmental biology, Harris' findings may have far-reaching implications.

"The repair of neural tissue is a very challenging field. In order to make progress, it is often helpful to understand more about how the brain is originally made, then try to recreate that in an injured brain. Scientists at Cambridge's Brain Repair Centre are using ideas from neural development in this context," said Harris. "A better understanding of the control and regulation of cell proliferation is also highly relevant to research into neural-origin cancers. The more we understand retinal development, the more information we can feed into the development of novel therapies."

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