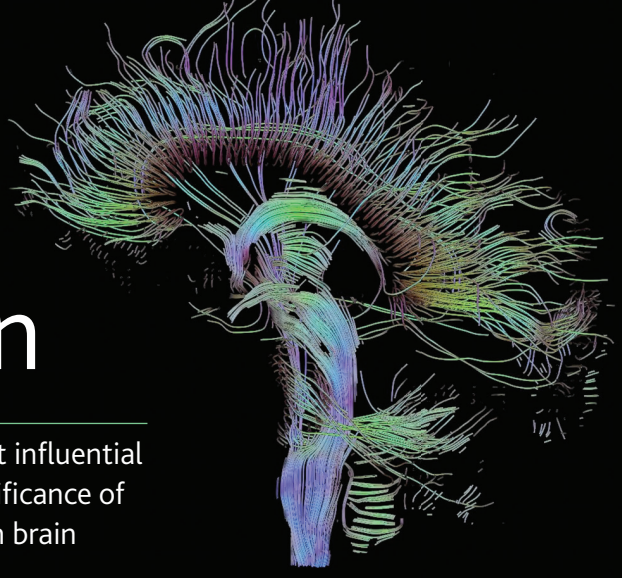


Rewiring the brain

Professor Jerold Chun, listed among Thomson Reuter's most influential minds, introduces his research on the potential adaptive significance of DNA content variation and genomic mosaicism in the human brain

How does your research on neuronal DNA content variation (DCV) in individuals advance the understanding that virtually all somatic cells contain identical amounts of genomic DNA?

A textbook description of cells in our body is that each somatic cell contains identical DNA. The discovery that normal neurons in the same individual could vary somatically at the level of single cell genomes proved the 'constant genome for all cells' description to be inaccurate. DCV encompasses a universe of genomic variability within and amongst single neurons (and other brain cells) that have been and are being documented. We now use DCV as a catch-all term that includes all changes capable of altering DNA content.

In an analysis of lagging chromosomes during neuroblast mitosis, you identified genetic alterations including hyperploidy, monosomy and trisomy. Is there a functional correlation between these chromosomal variations and aneuploid cortical cells?

Answers to this question are still emerging. It is notable that one of the clearest ways to alter brain function is through constitutive aneuploidy – like Down syndrome (three copies of chromosome 21 in all cells of one person) – which negatively alters brain function. However, mosaic aneuploidy occurs normally and involves many different chromosomes, suggesting that it could have beneficial roles in normal brain organisation and function. Indeed, aneuploid neurons can be integrated into brain circuitry, show altered gene expression and demonstrate preferential maintenance or removal based upon general forms of aneuploidy, all of which point to functional consequences.

Could you elucidate your group's suggestion that an important fraction of cells within the brain dies due to defects in chromosome numbers?

There appear to be differential susceptibilities to cell death or survival, at least during developmental periods based upon forms of aneuploidy. We don't completely understand these mechanisms, but it seems that developing neurons with many chromosome gains and/or losses are normally eliminated, whereas those cells with milder gains and losses are maintained, at least during development. This indicates that not all forms of aneuploidy are functionally equivalent; that is, they have functional significance that allows their removal or maintenance as the brain forms.

What is the significance of discovering that DCV within individual human brains showed regional variation?

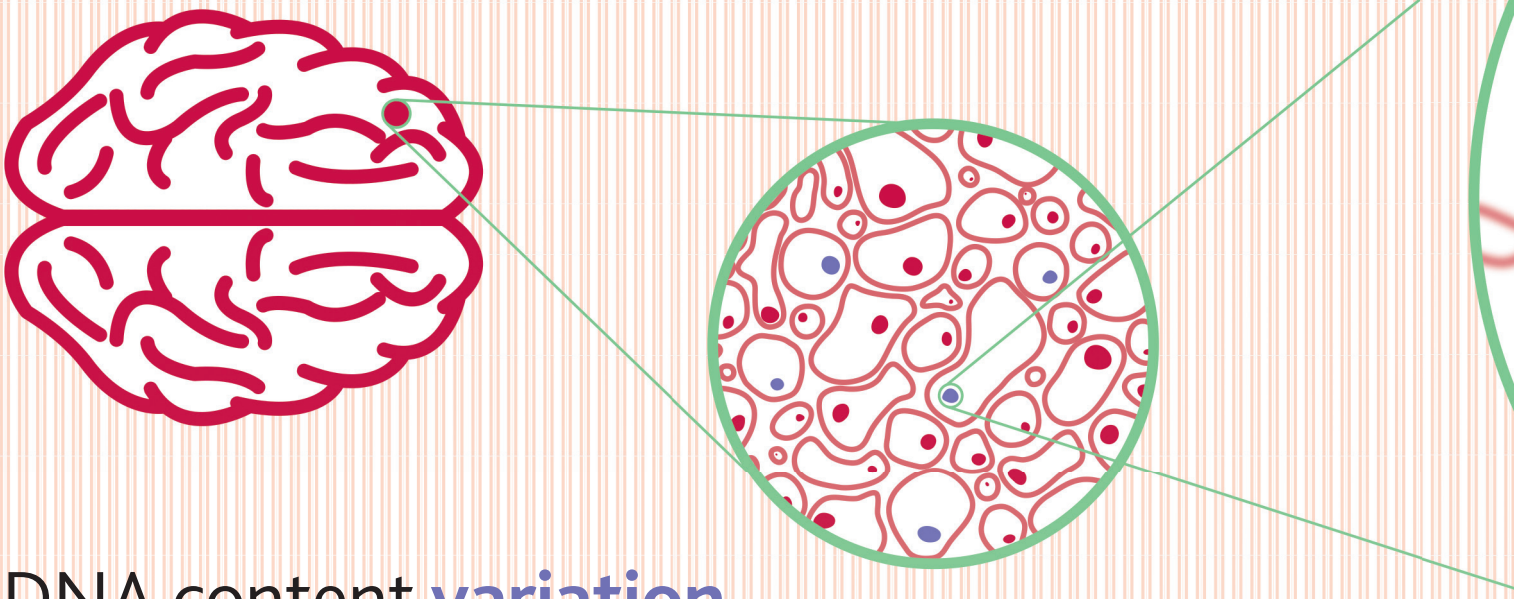
Early studies demonstrating genomic mosaicism through aneuploidy led to searches for other forms, and the simplest prediction of alterations in the genome is that it should be possible to detect changes in overall DNA content using flow cytometry. Since we observed both gains and losses of chromosomes, this would suggest the equivalent in DNA content, and this was indeed observed. However, patterns of gains and losses were not in concert with what we know is the more uniform presence of aneuploidies in the neuraxis – at least based on limited sampling data obtained by hybridisation-based techniques – and, therefore, it was fairly certain that additional forms of DNA gains and losses are occurring. This has been borne out by recent data showing copy number variants in single neurons – sub-chromosomal gains and losses of DNA – through the use of single-neuron sequencing that is being pursued by several groups, including our own. The fact that DCV shows regional variation underscores non-random processes that we believe are tied to functional aspects of the brain.

Can you provide some insight into the main objectives of your research on site-specific recombination (RAG-1 and RAG-2) and its role in genetic mobility and gene assembly? How did these investigations influence your subsequent work?

The RAG work preceded and stimulated a search for diverse genomic changes in cells of the brain, particularly neurons, including effects on cell death that are a hallmark of immunological cell selection. As part of the VDJ recombinase RAG-1 and RAG-2 expression in the brain could have implicated similar VDJ-like mechanisms, however, only RAG-1 is expressed. What it is doing in the brain remains unclear; the presence of other genes related to the recombination process – so-called non-homologous end-joining factors – do affect developing brain cell survival and point to genome-altering processes taking place within cells of the brain. The proven existence of many forms of DCV must involve DNA-altering enzymes and related molecules that increase or decrease DNA, and limit the changes to discrete genomic regions.

How will your findings impact knowledge of brain function?

The existence of myriad genomically distinct neurons – and likely other brain cell types that have their own forms of alterations – indicates that the brain is not only phenotypically diverse and complex, but that the diversity and complexity extends to individual cellular genomes. It is very likely, although not yet completely proven, that the forms and extent of genomic heterogeneity contribute to phenotypic heterogeneity. This is germane to development, function and disease. Every gene thus far identified in the brain could theoretically be affected by DCV and genomic mosaicism.



DNA content variation

A team from **The Scripps Research Institute**, USA, is making groundbreaking advances in understanding the human brain and, specifically, its genomic composition at the level of an individual's single cells

ONE OF THE fundamental pillars of biology has long been that all human cells belonging to a single individual (excluding gametes) share the same DNA, wherein a person's genetic makeup, known as the genotype, is constant throughout the body. However, this traditional paradigm is being challenged by research teams worldwide. In fact, studies have now identified neural cells throughout the neuraxis with somatically generated changes in DNA sequence, signifying that the vertebrate brain can be genomically heterogeneous.

Brain cells display DNA content variation (DCV), which manifests itself through an increased range of DNA content within cell populations and single cells, encompassing DNA sequence changes produced by mosaic aneuploidy, copy-number variation (CNV) and other DNA-altering forms. The brain is thus a genomic mosaic.

A group of researchers at The Scripps Research Institute, USA, led by Professor Jerold Chun, has carried out pioneering studies that first identified and defined forms of genomic mosaicism – an umbrella term that is relatively new to biology. It encompasses the range of processes and patterns producing genomically diverse neurons and other cell types in the brain of a single individual.

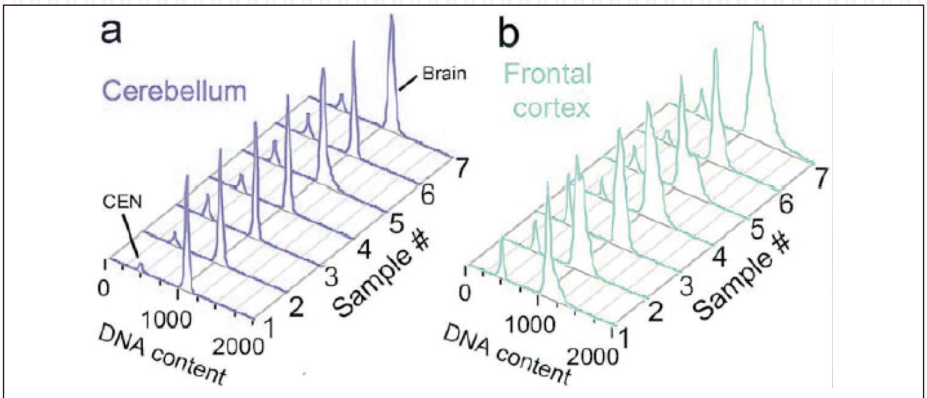
BRAIN DEVELOPMENT

In the 1990's Chun's team identified programmed cell death occurring in proliferating populations of the embryonic brain – a novel observation at the time. "Many other laboratories had defined cell death through detection of DNA fragmentation," Chun elucidates. "We increased the sensitivity and applied new approaches, validated with model cell death systems such as the small intestine, which combined with other controls, led to the identification of embryonic brain cell death." These studies supported variable susceptibilities of developing progenitor cells to counter cell death, possibly involving genomic alterations, based upon a dying cell's fragmented genome. These studies led to Chun's subsequent work demonstrating the existence of non-identical genomes amongst brain cells, resulting in current efforts at more deeply interrogating single-cell genomes and understanding the consequences of the mosaic sequence changes.

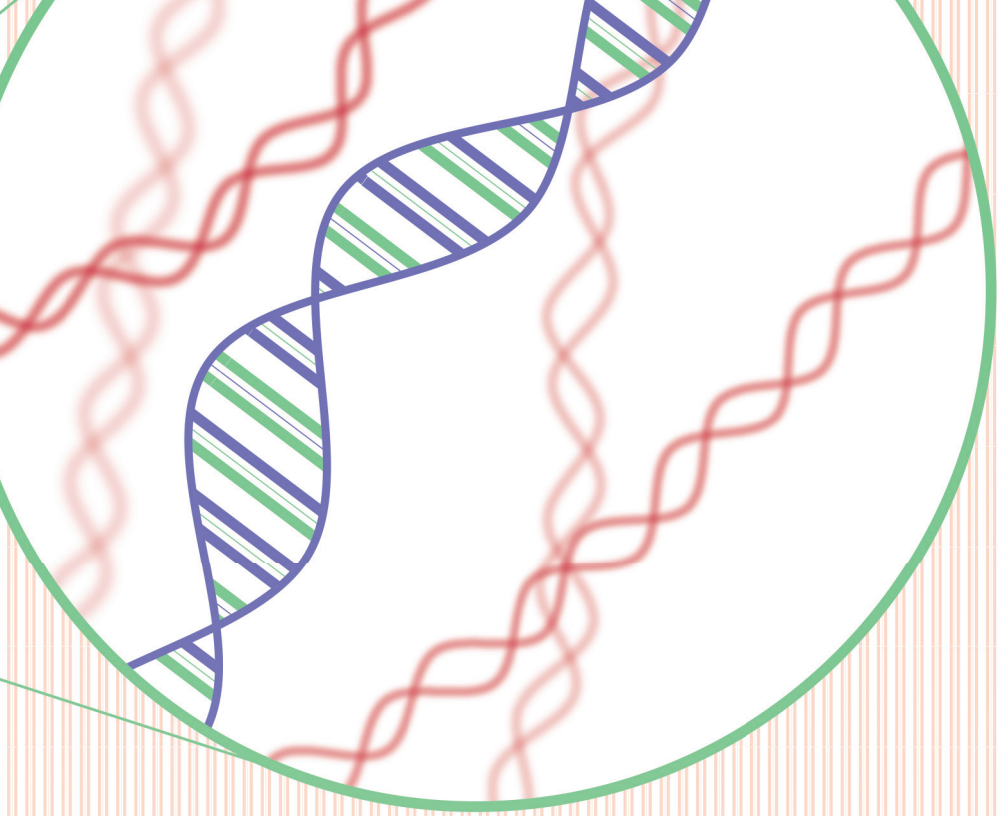
THE PURPOSE OF MOSAICISM

The Scripps team is now endeavouring to understand the functions of genomic mosaicism by examining the consequences and mechanisms underlying DCV, particularly mosaic aneuploidies and CNVs. "Mosaic aneuploidy – along with other DCV forms – occurs normally and involves many different chromosomes, suggesting that it could have beneficial roles in typical brain organisation and complex function," explains Chun. Evidence underscoring an adaptive purpose to genetic mosaicism is that DCV is differentially present throughout the brain. The contributions made by the researchers thus far demonstrate that the presence of brain cells with differing DNA is significant and more prominent in different parts of the brain. More recently, genomic states have been associated with embryonic brain cell death or survival. Now, the group is endeavouring to gain further functional insights relevant to brain development, normal processes and disease.

The importance of this research, and the potential implications and application of



DNA content variation (DCV) varies in different parts of the brain and within an individual brain (large peaks). Note the different shapes of the large peaks between the cerebellum and cortex, and within samples from each region, demonstrating different distributions of genomically distinct cells.



understanding this phenomenon are huge: "Every gene thus far identified in brain function could theoretically be affected by DNA content variation and genomic mosaicism," Chun notes. "Such diversity could underlie physiological differences including personality and behaviour, as well as brain diseases, particularly those unlinked to families: the so-called sporadic or idiopathic varieties of brain diseases that currently lack a cohesive, mechanistic understanding and that represent the overwhelming majority of neurological and psychiatric disease presentations."

A COMPLEX OPPORTUNITY

Research by Chun and his colleagues has shown that the brain is not only impressively complex at the structural level, but also at the genomic level. The team is now utilising cutting-edge techniques to understand effects of genomic mosaicism on brain function: "By somatically changing single neuronal (and other brain cell) genomes, we can consider new ways of forming brains and customising or sculpting their functions and capabilities," highlights Chun. The most exciting potential applications of an improved understating of the intricacy involved will be medical, with sufferers of idiopathic brain diseases potentially benefiting from future treatments arising from this enhanced understanding.

The ability to detect diverse types of DNA content variation in the brain has improved with the advent of innovative methodologies and detection technologies. "Virtually every modern approach to understanding the genome was and is being directed at the problem," explains Chun. A relatively early technique – developed by Thomas Reid at the National Institutes of Health (NIH) – called spectral karyotyping (SKY), first enabled the researchers to paint individual chromosomes in order to illustrate loses and gains of entire chromosomes, and define aneuploidy in neural progenitor cells. Newer techniques involving the use of single-cell sequencing technologies are being utilised by the Scripps team.

Though the discoveries made by the group are already exciting, research into genomic mosaicism and DNA content variation is in its infancy. Every new discovery raises more questions and it is certain that the full extent of genomic mosaicism and its associated mechanisms in the human brain represent a small universe to be understood. Within this context, the work that Chun and his colleagues will pursue in the coming years will be essential from both academic and medical perspectives. Therefore, although the outputs of this research are difficult to predict, the potential applications emerging from a comprehensive understanding of single-cell genomes in brain function and variation are extremely promising.

Studies have now identified neural cells throughout the neuraxis with somatically generated changes in DNA sequence, signifying that the vertebrate brain can be genomically heterogeneous

INTELLIGENCE

GENOMIC MOSAICISM IN SINGLE NEURONS OF THE BRAIN: DNA CONTENT VARIATION (DCV) AS A SUBSTRATE FOR DIVERSITY AND COMPLEX FUNCTION

OBJECTIVE

To gain greater insight into genomic mosaicism, whereby myriad neurons (and other cell types) in the brain of a single individual possess non-identical genomes, and understand its origins and consequences.

KEY COLLABORATORS

Dr Kun Zhang, University of California, San Diego, USA

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JEROLD CHUN is Professor of Molecular and Cellular Neuroscience at The Scripps Research Institute. The Chun Laboratory's overarching goal is to understand the brain – how it develops, carries out complex tasks, and is affected by diseases. This is being pursued through two scientific areas that have both basic science and therapeutic implications: genomic mosaicism among single brain cells – especially neurons – whereby cells from the same individual can vary at the genomic level; and lysophospholipid receptor signalling, involving small lipids that act as extracellular chemical signals. Within these areas, the group has placed a particular emphasis on translating basic science research into understanding and potential treatments for brain diseases, with current efforts aimed at hydrocephalus, multiple sclerosis (MS), Alzheimer's disease, and other neurodegenerative diseases. The entry of fingolimod – a compound that acts through a subgroup of lysophospholipid receptors – into the clinical treatment of MS, underscores the real-life relevance and future potential of these lines of research.