



Cellular and Molecular Explorations of Anthropogeny

Public Symposium • Friday, September 29, 2017

Chairs:

Fred H. Gage, Salk Institute & **Svante Pääbo**, Max Planck Institute for Evolutionary Anthropology

Sponsored by:

Center for Academic Research and Training in Anthropogeny (CARTA)

With generous support from:

The G. Harold and Leila Y. Mathers Charitable Foundation

ABSTRACTS

Cellular and molecular mechanisms that differentiate human and non-human neural development

Fred H. Gage, Salk Institute

Understanding the evolutionary mechanisms underlying expansion and reorganization of the human brain is essential to comprehend the emergence of the cognitive abilities typical of our species. Comparative analyses of neuronal phenotypes in closely related species (*Homo sapiens*: human; *Pan troglodytes*: chimpanzees; and *Pan paniscus*: bonobos) can shed light onto neuronal changes occurring during evolution, the timing of their appearance and the role of evolutionary mechanisms favoring a particular type of cortical organization in humans. The availability of post-mortem brains of endangered primates is limited and often does not represent important species-specific developmental hallmarks. We used induced pluripotent stem cell (iPSC) technology to model neural progenitor cell and neurons both functionally and genomically. This presentation provides a cellular and molecular analysis of comparative neural development in closely related hominids. The strategy proposed here lays the groundwork for further comparative analysis between human and non-human primates and opens new avenues for understanding the differences in the neural underpinnings of cognition and neurological disease susceptibility between species.

Understanding primate brain development using stem cell systems

Rick Livesey, Gurdon Institute, University of Cambridge

Variations in cerebral cortex size and complexity are thought to contribute to differences in cognitive ability between humans and other animals. We are using primate stem cell systems to understand the cellular and molecular mechanisms underlying species differences in cerebral cortex development. Directed differentiation to cerebral cortex of human and non-human primates pluripotent stem cells (PSCs) in adherent two-dimensional (2D) and organoid three-dimensional (3D) culture systems enables comparative studies of neurogenesis in different species, including chimpanzee and macaque. Using these approaches, we have identified mechanisms for controlling cerebral cortex size that are regulated cell-autonomously, suggesting that primate cerebral cortex size is regulated at least in part at the level of individual cortical progenitor cell clonal output. We are using a range of methods, including single cell RNA sequencing and comparative genomics, to identify candidate genes and pathways that regulate the output of each cortical progenitor cell, and thus contribute to species differences in brain size.

Human-specific genes and neocortex expansion in development and evolution

Wieland Huttner, Max Planck Institute for Molecular Cell Biology and Genetics

The expansion of the human neocortex, which constitutes a basis for our cognitive abilities, is due to an increased abundance and proliferative capacity of neural stem and progenitor cells (NPCs) during fetal cortical development. Specifically, NPCs in a secondary germinal zone called the subventricular zone, referred to as basal progenitors (BPs), are thought to be key for neocortex expansion. Here, a particularly important role has been attributed to one specific BP type called basal (or outer) radial glia (bRG).

In search for human-specific genome changes underlying neocortex expansion, we have found that the human-specific gene *ARHGAP11B* is specifically expressed in bRG and the NPCs from which bRG are derived. When expressed in embryonic mouse neocortex, *ARHGAP11B* causes amplification of BPs and is able to induce folding of the embryonic mouse neocortex, which is normally smooth. The ability of *ARHGAP11B* to amplify BPs is based on a single C-to-G base substitution that, due to a reading-frame shift resulting from altered mRNA splicing, is ultimately responsible for the appearance of a novel, human-specific 47-amino acid sequence in *ARHGAP11B* that is thought to be essential for BP amplification.

Cellular and molecular features of human brain expansion and evolution

Arnold Kriegstein, UC San Francisco

The developing human brain contains a huge number of cells whose identities have not yet been fully explored but whose specific molecular and functional features lead to the development of human specific abilities. We are using single cell approaches to establish an integrative definition of cell types in the developing human neocortex. Our single cell genomics analysis has revealed the molecular identity of a key human progenitor cell type, termed an outer radial glia cell (oRG). The developing human cortex contains a massively expanded progenitor region that is enriched in oRG cells that are thought to contribute to the developmental and evolutionary increase in cortical size and complexity of the human brain. We sequenced mRNA from single human progenitor cells and found that oRG cells preferentially express genes involved in growth factor signaling and self-renewal pathways, suggesting that oRG cells establish a self-sustaining proliferative niche. Using single cell clonal lineage analysis, we found that oRG cells can generate hundreds of daughter neurons, establishing the extensive proliferative and neurogenic capacity of this cell type. Surprisingly, we also discovered that the mTOR signaling pathway, known to promote cell growth and proliferation in a wide variety of cell types, is selectively active in oRG cells. This finding highlights a previously unappreciated cellular pattern of selective vulnerability and may have implications for our understanding of human diseases associated with MTOR pathway mutations, such as autism.

By using novel markers that reveal the morphology of radial glia cell subtypes, we discovered that the radial glial scaffold, which has classically been viewed as a continuous structural framework upon which the cortex develops, becomes a discontinuous pathway entirely constructed by oRG cell fibers. This transformation occurs partway through human brain development and results in a different lineage for upper cortical layer neurons compared to deeper layer neurons. This developmental event may underlie primate-specific features of upper cortical layer neurons that have been related to higher cognitive functions in humans.

Together, our results highlight cellular features of human brain development that are not represented in animal models and may reflect human or primate-specific evolutionary adaptations. These findings also provide a roadmap for interpreting laboratory models of human brain development and evolution.

Making faces: Regulatory evolution and variation in the human neural crest

Joanna Wysocka, Stanford University

From Galapagos finches to anteaters, the remarkable diversity of craniofacial structures within the vertebrate species is a testament to the plasticity of development and resourcefulness of evolution. While craniofacial development requires interactions between multiple embryonic cell types, Cranial Neural Crest Cells (CNCCs) play a major role in establishing the central plan of facial morphology as well as determining its species-specific variation. I will discuss an approach we termed “cellular anthropology” in which *in vitro* differentiation models using hominid iPSCs can be effectively applied to studies of both basic physiology as well as evolutionary questions. Specifically, I will focus on using cellular anthropology to understand how sequence variation in human CNCC regulatory elements produce quantitative and cell type-restricted transcriptional changes that can mediate morphological evolution and individual variation of the craniofacial form.

Reconstructing uniquely human cellular functions using ancient genomes, mice and stem cells

Svante Pääbo, Max Planck Institute for Evolutionary Anthropology

Modern humans differ from all other primates, and now extinct hominins, in that they develop culture and technology that are highly complex and change rapidly. By comparing the genomes of apes, Neandertals and other extinct human forms to those of present-day humans, we can identify genetic changes that are shared among all, or almost all, present-day humans and that are potentially important for cognitive abilities. We will introduce these changes into the genomes of cells that we will differentiate into neurons and brain-like structures in tissue culture. We have introduced some such changes into mice and into stem cells and are investigating their functional consequences.

One example is FOXP2, a transcription factor involved in the acquisition of language that carries two amino acid substitutions that are unique to humans. Mice carrying a humanized version of *Foxp2* show increased procedural learning and have altered ultrasonic vocalizations, which have been linked to changes in cortico-basal ganglia circuits. By analyzing gene expression in single cells, we find that *Foxp2* is expressed in a subset of medium spiny neurons in the striatum and pyramidal neurons in layer 6 of the frontal cortex. We compare these *Foxp2*-expressing cells in wild type and humanized mice to understand how the change of two amino acids alters cell type-specific gene regulatory networks. In parallel, we are establishing an *in vitro* system to model the effect of ancestralizing the FOXP2 gene in human neurons. Using the latter system, we are also studying human-specific changes in metabolic enzymes that may influence brain function, and changes in genes that are important for how nerve cells establish contact with each other and communicate.

Molecular and cellular mechanisms underlying human-specific evolution of cortical connectivity

Franck Polleux, Columbia University

Two prominent hallmarks of the human brain are the prolonged maturation time of neuronal circuits and a significant increase in cortical neuron connectivity. These features have been hypothesized to underlie the emergence of higher cognitive functions in modern humans. However, little is known about the molecular changes that have led to the emergence of human-specific traits of cortical development and function. Our attention has recently focused on gene duplications that are unique to the human genome. One such gene is SRGAP2: the ancestral copy, SRGAP2A, promotes excitatory (E) and inhibitory (I) synapse maturation and limits the density of both E and I synapses made onto cortical pyramidal neurons (Charrier et al. Cell 2012; Fossati et al. *Neuron* 2016). Partial duplication of SRGAP2A

resulted in a human-specific paralog, SRGAP2C, which binds to and inhibits SRGAP2A function. Deletion of SRGAP2A or expression of SRGAP2C in mouse cortical pyramidal neurons leads to the emergence of phenotypic traits characterizing human cortical neurons, including increased E and I synaptic density and a protracted period of synaptic maturation. However, how this increased density of synapses and prolonged maturation affects the structure and function of cortical circuits remains unknown and constitutes the main goal of our current project. I will present some new results probing changes in circuit structure and function upon SRGAP2A loss-of-function or ‘humanization’ of SRGAP2C expression in mouse cortical circuits. Our results provide new insights into the significance of the emergence of human-specific SRGAP2C gene on brain evolution by defining its impact on synaptic organization and circuit function.

Evolution and tinkering: Nuclear structure and primate-specific gene regulation
Martin Hetzer, Salk Institute

Nuclear pore complexes (NPCs) emerged as nuclear transport channels in eukaryotic cells ~1.5 billion years ago. While the primary role of NPCs is to regulate nucleo–cytoplasmic transport, recent research suggests that certain NPC proteins have additionally acquired the role of affecting gene expression at the nuclear periphery and in the nucleoplasm in metazoans.

We provide evolutionary and cell-biological evidence for the presence of a previously undetected soluble form of the transmembrane nucleoporin (Nup) *Pom121*. This variant that we call *sPom121* lacks the nuclear membrane-anchoring domain and thus does not localize to the NPC. Instead, *sPom121* colocalizes and interacts with nucleoplasmic *Nup98*, a previously identified transcriptional regulator, at gene promoters to control transcription of its target genes in human cells.

sPom121 arose in the last common ancestor of hominoids from a rearrangement of genomic sequences that generated a new transcriptional start site, exhibiting a classic example of what Jacob (1977) called “molecular tinkering.” According to his idea, the appearance of a new molecular function is generated by alterations of pre-existing ones. In the context of the NPC, it appears that the adaptive advantage of generating *sPom121* as a soluble NLS-containing regulator appears to lie in the potential uncoupling of Nup-mediated gene regulation from the spatial constraints of NPC-mediated genome regulation at the nuclear periphery. This uncoupling allows the expansion of *Pom121*'s role in transcriptional regulation without compromising its role at the NPC, providing an elegant demonstration of the relief from the inherent antagonistic pleiotropy of encoding two important functions in the same Nup protein.

The evolution of human-specific genes by duplication
Evan Eichler, University of Washington School of Medicine

Huxley and Darwin were among the first to appreciate the close evolutionary relationship of humans and other African great apes but also to ponder what genetic changes might make us human. Initial comparisons of human and chimpanzee genes, however, showed little difference (>99% identical) despite the numerous adaptations that must have occurred on the human lineage. Recent studies of more complex regions of our genome have revealed hotspots of rapid and dramatic evolutionary change. Embedded within these regions are hundreds of new duplicate genes, several of which appear to be important in unique human-specific neuroanatomical adaptations, including the expansion of the neocortex and increase in synaptic connectivity. Paradoxically, this genetic complexity has led to a high background rate of copy number mutations causing childhood diseases (e.g., autism, intellectual disability, and epilepsy) suggesting that human-specific genes and increased disease burden are tightly linked.