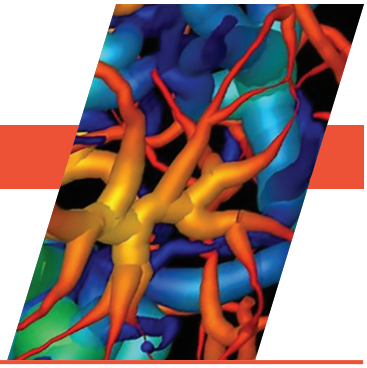
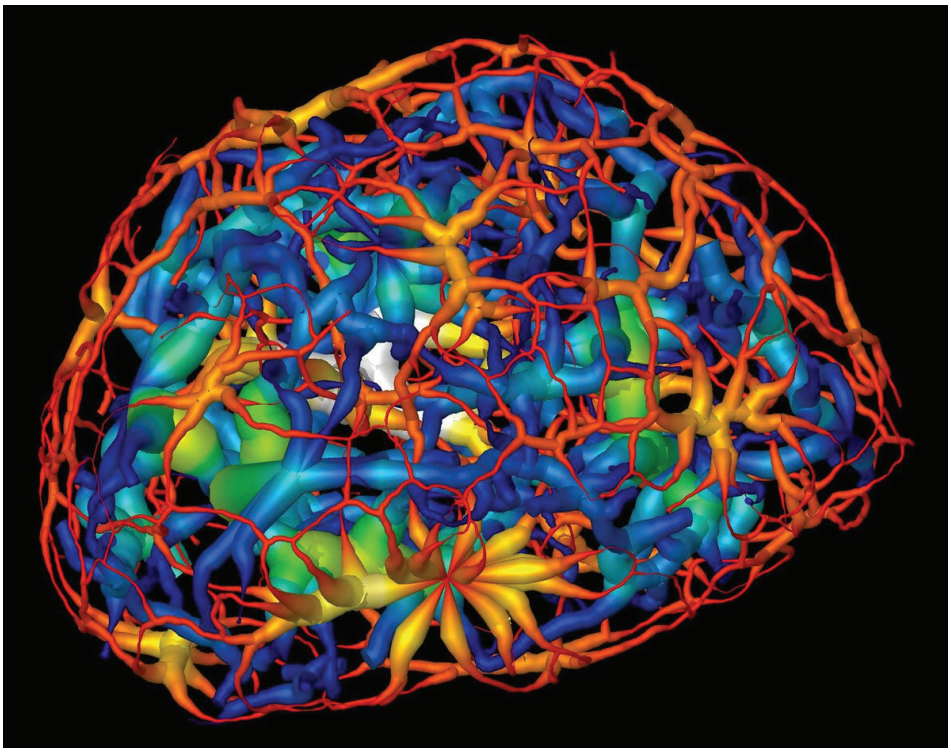
BIOLOGICAL SCIENCES

Mapping the Migration of Genetic Material





This computer rendering shows the skeletonized structure of heterochromatin (red represents a thin region while white represents a thick region), a tightly packed form of DNA, surrounding another form of DNA-carrying material known as euchromatin (dark blue represents a thin region and yellow represent the thickest) in a mouse's mature nerve cell. (Credit: Berkeley Lab, UCSF)

Researchers have used a powerful soft x-ray microscope at the ALS to capture tomographic images of the genetic material in the nuclei of nerve cells at different stages of maturity. This study, the first quantitative analysis of nuclear organization in intact mammalian cells, has generated detailed 3D visualizations that show an unexpected connectivity in the genetic material, called chromatin. The results could help us understand how the patterning and reorganization of chromatin relate to the specialization of a stem cell's function as specific genes are activated or silenced.

During cell division, chromatin, which consists of DNA, RNA, and other proteins, is compacted to form the chromosomes that pass along an organism's genetic fingerprint to newly formed cells. The distribution of chromatin in the nucleus differs between cell types and developmental stages, suggesting that nuclear organization serves regulatory functions. However, understanding the logic of nuclear architecture and how it contributes to stem-cell differentiation remains challenging. Until now, it has only been possible to image the nucleus indirectly, by staining it, in which case the researcher has to take a leap of faith that the stain was evenly distributed. Chromatin is notoriously sensitive to chemical stains and other chemical additives that are often used in biological imaging to highlight regions of interest in a given sample.

In this work, researchers used soft x-ray microtomography at ALS Beamline

A Step Toward Precision Medicine

The way DNA is organized in a cell nucleus can affect genetic function. The ability to map this 3D organization is an important step toward targeted, individualized treatment of genetic disorders. For example, one of the precursors to Alzheimer's disease, which attacks the brain's nerve cells, is a loss of smell, so understanding this connection to olfactory nerve cells could perhaps serve as a diagnostic tool and perhaps unlock a deeper understanding of the degenerative disorder.

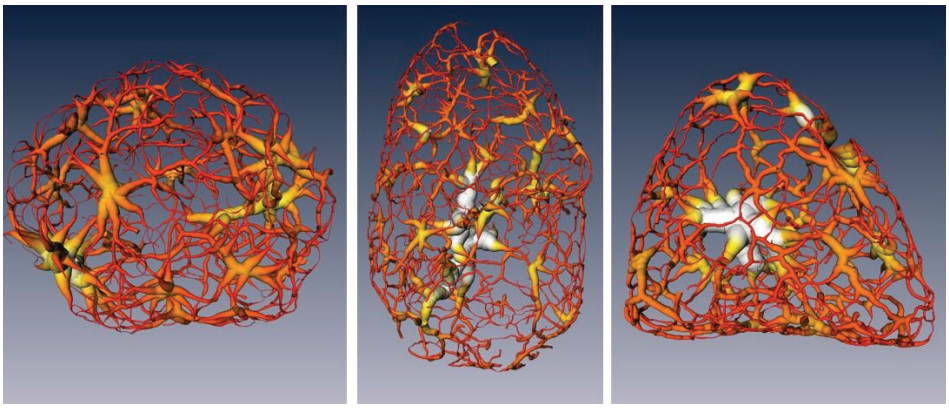
One aim of the latest study was to gain new insight into gene expression in mice specific to olfactory genes. Mice have about 1,500 genes related to smell. Each olfactory nerve cell expresses just one of these olfactory genes to produce a receptor that recognizes a related set of odors. The many receptors in a mouse's nasal cavity allow it to detect a wide range of smells.

In this work, Le Gros et al. are trying to understand how the reorganization of genetic material in the cell nucleus affects gene expression. No one has been able to study this at the human level yet. This research will hopefully lead to new insights about diseases and disorders that relate to gene expression. Already, the study's results are being incorporated into models of cell development.

2.1 (part of the National Center for X-Ray Tomography) to record a series of images from mouse olfactory nerve cells in three separate stages of development. The technique, which is unique to the ALS, provides a new way of looking at the nucleus without the need to chemically treat the cell, allowing visualization of intact cells in a near-native state at a resolution of about 50 nanometers.

Frozen cells at each stage were imaged from dozens of different angles, and each set of 2D images was used to calculate a 3D reconstruction detailing the changing chromatin formations in the nucleus. The images were collected using soft x-rays within the "water window" (284–543 eV), where biomolecules absorb x-rays an order of magnitude more than the surrounding water. The absorption is linear with biochemical composition and concentration, generating a unique x-ray linear absorption coefficient measurement for each voxel (3D pixel analog). Thus, the researchers were able to visually distinguish between different types of chromatin: heterochromatin, due to an increased biomolecular concentration, appears darker than euchromatin in computer-generated tomographic orthoslices (virtual sections) through the nucleus.

The results showed that chromatin compaction increases as the cell matures, and that condensed chromatin moves to the nuclear core during differentiation. Surprisingly, while it was previously thought that the chromatin existed as a series of disconnected islands, the results showed how the chromatin was compartmentalized into two distinct regions of "crowding" that form a continuous network throughout the nucleus. Also, based on comparison of these results to those of similar cells in which the gene for HP1β (a heterochromatin binding protein) had been inactivated ("knocked out"), the researchers concluded that HP1β regulates the reorganization of chromatin in mature neurons.



These renderings show heterochromatin as it exists in a mouse cell's nucleus at different stages of cell development: a multipotent stem cell (left), a neuronal progenitor (middle), and a mature nerve cell (right). (Credit: Berkeley Lab, UCSF)

Soft x-ray tomography provides a powerful method to study chromatin and nuclear architecture in vivo. There is no need for chemical fixation and sectioning, preventing a plethora of artifacts introduced either by fixatives or by visualization of only thin sections. With the proven success of the imaging technique, the researchers believe it is possible to perform statistical analyses based on large collections of cell nuclei images sorted by different stages of development. Coupled with other types of imaging techniques, researchers hope to isolate individual gene-selection processes in upcoming work.

Publication about this research: M.A. Le Gros, E.J. Clowney, A. Magklara, A. Yen, E. Markenscoff-Papadimitriou, B. Colquitt, M. Myllys, M. Kellis, S. Lomvardas, and C.A. Larabell, "Soft x-ray tomography reveals gradual chromatin compaction and reorganization during neurogenesis in vivo," *Cell Reports* **17**, 2125 (2016). dx.doi.org/10.1016/j.celrep.2016.10.060

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