Submitter Name: Sergei Grigoryev Submitted Email: sag17@psu.edu

Cryo-electron tomography reveals the inner structure of nucleosome condensates in chromatin of epigenetically modified mouse retina

Evgenya Popova[1], Colin Barnstable[1], Carson Purnell[2], Matthew Swulius[2], and Sergei Grigoryev[2]

[1]Department of Neural and Behavioral Sciences;
[2]Department of Biochemistry and Molecular Biology, Penn State University, College of Medicine, Hershey PA 17033.

Heterochromatin spreading is critical for transcriptional reprogramming during neuronal cell differentiation and restricting cell regeneration. Targeting heterochromatin by epigenetic modifiers that act by changing genome structure and functions without altering the DNA is a powerful approach that can be applied to treatment of neurological disorders for which gene therapy is not feasible. Strikingly, in a blind mouse model of retinitis pigmentosa affecting mature retina, injections of epigenetic histone modifiers, HDAC1 and LSD1 inhibitors, can reverse retina degeneration and partially restore vision. We hypothesized that these epigenetic modifiers inhibit nucleosome condensation leading to a partial heterochromatin opening and reactivation of genes preventing retina maturation. To reveal the nanoscale anatomy and mechanism(s) underlying chromatin condensation and its reversal by HDAC and LSD1 inhibitors, we have combined Cryo-electron tomography with a newly developed deep-learning AI-based denoising and quantitative stereological analysis. Cryo-ET imaging revealed the following features specific to the condensed neuronal chromatin: formation of large nucleosome condensates containing a mixture of randomly oriented and clusters of stacked nucleosomes; folding of nucleosome into irregular zigzag nanoparticles rather than continuous fibers; treatment by the epigenetic modifiers promotes face-to-face nucleosome stacking and overall higher-order folding typical of proliferating cells; in control retina, the stacking interactions are reduced with increased propensity to interact *in-trans* thus promoting chromatin self-association and condensation. We propose that the nucleosome condensates are formed via interdigitation between nucleosome disks and this process can be targeted by epigenetic modifiers to promote cell regeneration.

This work is supported by NIH grant R21 DA056343