



GRADUATE SCHOOL OF BIOMEDICAL SCIENCES

BIOCHEMISTRY AND MOLECULAR PHARMACOLOGY

Ph.D. THESIS DEFENSE

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MENTOR: Craig L. Peterson, PhD

THURSDAY, 8/20/2015 10:00 a.m.

Biotech 2, 3rd floor seminar room

"The shape of silence: the solution-state conformation of SIR heterochromatin"

Heterochromatin is a silenced chromatin region essential for maintaining genomic stability in eukaryotes and for driving developmental processes in higher organisms. A hallmark of heterochromatin is the presence of specialized architectural proteins that alter chromatin structure to inhibit transcription and recombination. Although it is generally assumed that heterochromatin is highly condensed, surprisingly little is known about the structure of heterochromatin or its dynamics in solution. In budding yeast, heterochromatin assembly at telomeres and the HM silent mating type loci requires the Sir proteins: Sir3, believed to be the major structural component of SIR heterochromatin, and the Sir2/4 complex, responsible for SIR recruitment to silencing regions and deacetylation of lysine 16 of the histone H4 tail, a mark associated with active chromatin. A combination of sedimentation velocity, atomic force microscopy, and nucleosomal array capture was used to characterize the stoichiometry and conformation of SIR nucleosomal arrays. The results indicate that Sir3 interacts with nucleosomal arrays with a stoichiometry of two Sir3 monomers per nucleosome, and that Sir2/4 may additionally bind at a ratio of one per nucleosome. Despite Sir3's ability to repress transcription *in vivo* and homologous recombination *in vitro* in the absence of Sir2/4, Sir3 fibers were found to be significantly less compact than canonical magnesium-induced 30 nanometer fibers. However, heterochromatin fibers composed of all three Sir proteins did adopt a more condensed, globular structure. These results suggest that heterochromatic silencing is mediated both by the creation of more stable nucleosomes and by the steric exclusion of external factors.

Mentor(s)

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