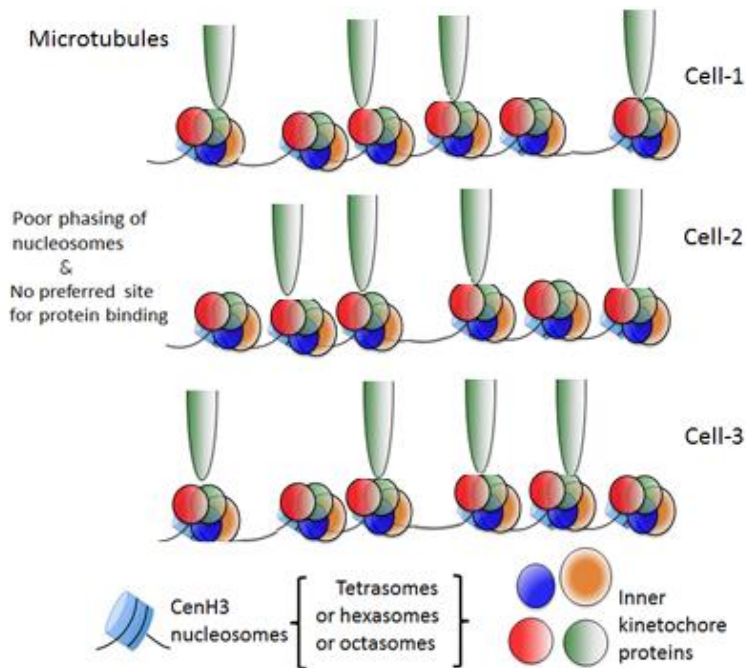


# Journey to the center of the chromosome

December 20, 2015

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A model for regional centromeres of fission yeast.

Image provided by Dr. Jitendra Thakur (Basic Sciences Division)

Cellular reproduction depends upon the faithful propagation of genetic material. Kinetochores are large protein complexes that anchor chromosomes to the microtubules that will physically pull them into daughter cells. They assemble at a specific location on each chromosome called the centromere. There is tremendous diversity in the structure and DNA sequence of centromeres across species, ranging from the ~125 base-pair point centromere of budding yeast to the holocentric centromeres of some plants and animals that span the length of the entire chromosome. The regional centromeres of the fission yeast *Schizosaccharomyces pombe* are a model for understanding the animal centromere, which is typically composed of megabase-pair sized arrays of highly repeated sequence that is refractory to sequence alignment and mapping. It is the organization of the fission yeast centromere that bears similarity to animal centromeres; it has a central domain composed of some unique sequence surrounded by the innermost repeats and, surrounding that, a pericentromeric region composed of more degenerative repeats. The pericentromere is highly heterochromatic, meaning its DNA is tightly wound around nucleosomes.

Most nucleosomes are identical, with two copies each of the histone H2A, H2B, H3 and H4 proteins, but there are a few variations that are important for DNA- and protein-based processes such as

transcription and kinetochore assembly, respectively. The histone H3 variant CenH3, also known as CENP-A, marks the centromeres of nearly all known eukaryotic organisms. Despite this staggering conservation, the localization of CenH3 nucleosomes within the centromere has been difficult to study without high-resolution methods. The precise localization of other important proteins that associate with the centromere, including those that are part of the kinetochore, has been even less studied. In order to get a better picture of centromeric protein organization, postdoctoral fellow Jitendra Thakur and staff scientist Paul Talbert, in the laboratory of Dr. Steven Henikoff (Basic Sciences), performed a thorough investigation of the localization of the centromeric histone variant CenH3 as well as the inner kinetochore proteins CENP-C, CENP-T, CENP-I using both native (N-ChIP) and crosslinking (X-ChIP) chromatin immunoprecipitation followed by sequencing. Their work was recently published in *Genetics*.

Previous mapping studies in *S. pombe* have revealed inconsistent results regarding the localization pattern of CenH3 nucleosomes. To resolve the inconsistencies, the authors performed CenH3 X-ChIP-seq and found that CenH3 was strongly enriched in the central domain compared to the pericentromere and the chromosome arms. Intriguingly, their X-ChIP-seq and N-ChIP-seq data revealed “ a nearly complete lack of CenH3 nucleosome phasing, in contrast to the precise phasing that we have seen for budding yeast and human centromeres, said Jitendra. This means that the centromeric nucleosomes do not localize across the central domain in the same type of consistent pattern that is seen for nucleosomes on the rest of the chromosome. Interestingly, we found that other inner kinetochore proteins also show no sequence preference, while respecting flanking pericentric boundaries, like a bowl of soup. Of particular interest to the kinetochore field is their finding that CENP-T binds to a range of DNA lengths while CENP-C and CENP-I appear to associate with DNA primarily through binding CenH3. The importance and function of CENP-T in kinetochore assembly has been only recently recognized and investigated.

The structure of the centromeric nucleosome has been a matter of debate in the field. Using a method that marks the precise position of histone H4 in every nucleosome in the genome, we have recently shown that CenH3 nucleosomes in budding yeast contain a single H4 molecule, reported Jitendra (see Henikoff et al 2014). Using the same method in fission yeast, Jitendra and her colleagues found that *S. pombe* CenH3 nucleosomes contain two H4 molecules, similar to ordinary H3 nucleosomes that occupy the rest of the genome.”

The fission yeast centromere serves as an important model system to understand the mechanism of chromosome segregation in higher eukaryotes. While some aspects of these findings are likely relevant across species, the irregular phasing of the centromeric nucleosomes themselves appears

specific to the non-repetitive central domain of *S. pombe* centromeres. Jitendra keenly observes that it will be valuable to exploit this to uncover the functional implications of phased versus unphased CenH3 nucleosomes at centromeres.”

[Thakur J, Talbert PB, Henikoff S](#). 2015. Inner kinetochore protein interactions with regional centromeres of fission yeast. *Genetics*. 201(2):543-61.

Also see:

[Henikoff S, Ramachandran S, Krassovsky K, Bryson TD, Codomo CA, Brogaard K, Widom J, Wang JP, Henikoff JG](#). 2014. The budding yeast Centromere DNA element II wraps a stable Cse4 hemisome in either orientation in vivo. *eLife*. 3:e01861.

[Henikoff JG, Thakur J, Kasinathan S, Henikoff S](#). 2015. A unique chromatin complex occupies young  $\alpha$ -satellite arrays of human centromeres. *Science Advances*. 1(1):e1400234.

This research was funded by Howard Hughes Medical Institute.