

chromosome structure have broad implications in studying effects of the geometry of nucleus on higher-order genome organization and nuclear functions. Here we describe a multi-chromosome constrained self-avoiding chromatin model for studying ensembles of structural genome models to understand the folding principles of budding yeast genome. We successfully generated a large number of model genomes of yeast under different geometrical constraints and found that spatial confinement of cell nucleus and molecular crowding in the nucleus are key determinants of the folding behavior of yeast chromosomes. Furthermore, the relative positioning of chromosomes and the interactions between them are found to be due to presence of nuclear landmarks such as centromere tethering to spindle pole body.

#### 2728-Pos Board B158

##### Torque Mediated Kinetics of CENP-A Nucleosomes Reveals Resilience to Tension

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In eukaryotic cells, DNA is wrapped around histone octamers, forming nucleosomes. In the centromere, the region of the chromosome that links sister chromatids, histone H3 is replaced by CENP-A (Centromere protein A). Since CENP-A chromatin is the point of contact between the microtubules/kinetochore complex and the rest of the chromosome, these regions endure very high forces. Whereas canonical nucleosomes unwrap at 3pN and disassemble at 15-20pN of force, the estimated forces applied by microtubuli are much higher. To investigate how CENP-A nucleosomes respond to externally applied forces and torque, we studied how CENP-A chromatin responds to defined stretching forces and torque generated by magnetic tweezers. With this technique, a single DNA molecule containing a few (up to 10) nucleosomes is tethered between a glass surface and a magnetic bead. By applying stretching forces at constant negative and positive supercoiling, the forces needed for disassembly of canonical H3 and CENP-A nucleosomes can be obtained and compared. By measuring the DNA end-to-end length as a function of applied rotations before and after CENP-A nucleosomes are removed from the DNA, the linking number of the nucleosomes can be determined. Interestingly, while inducing supercoiling at constant low (<0.5pN) forces, unlike canonical nucleosomes, CENP-A nucleosomes appear to respond to the applied torque. By further analyzing the structural stability of CENP-A nucleosomes under stretching force and torque, we hope to unravel the mechanism by which CENP-A nucleosomes resist disassembly during mitosis *in vivo*.

#### 2729-Pos Board B159

##### The Chd1 Chromatin Remodeler can Sense a Protein Bound at the Edge of the Nucleosome and is Sensitive to DNA Unwrapping

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Chromatin remodelers are essential for establishing and maintaining the placement of nucleosomes along genomic DNA. However, how remodelers respond to transcription factors and other bound factors that might influence chromatin organization is poorly understood. Here we use the Lac repressor to investigate how the Chd1 remodeler responds to a protein bound to the edge of a nucleosome. We found that Lac repressor effectively provided a barrier for nucleosome sliding by Chd1. This barrier did not require an absolute block in sliding, but instead was achieved through the bidirectional movement of nucleosomes, with a higher preference for sliding nucleosomes away from occupied Lac repressor sites. The presence of Lac repressor did not markedly diminish the affinity of Chd1 for nucleosomes, suggesting that Lac repressor is sensed after nucleosome sliding has been initiated. Nucleosome sliding rates were also reduced by DNA unwrapping, suggesting a mechanism by which Chd1 may indirectly sense factors bound at the edge of the nucleosome.

#### 2730-Pos Board B160

##### Mechanochemistry of Persistent Plasmid Movement

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The segregation of DNA prior to cell division is essential to the faithful inheritance of the genetic materials. In many bacteria, the segregation of the low-copy-number plasmids involves an active partition system composed of ParA ATPase and DNA-binding ParB protein, which stimulates the hydrolysis activity of ParA. Both *in vivo* and *in vitro* experiments have shown that the ParA/ParB system can drive the persistent movement of the plasmids in a directed fashion, just like a processive motor protein. However, the underlying mechanism

remains unknown. We have developed the first theoretical model on ParA/ParB-mediated motility. We establish that the coupling between the ParA/ParB biochemistry and its mechanical action works as a robust engine. It powers the directed movement of plasmids, buffering against the diffusive motion. Our work thus sheds light on a new emergent phenomenon, in which elaborate mechanochemical couplings of non-motor proteins can work collectively to propel cargos to designated locations, an ingenious way shaped by evolution to cope with the lack of a processive motor protein in bacteria.

#### 2731-Pos Board B161

##### Nucleosome Repeat Length Relates to the Gene Expression Level in Yeast

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We are investigating correlation between the DNA folding in 30-nm chromatin fiber and the level of gene expression. The 30-nm fiber is generally characterized by the nucleosome repeat length (NRL) - that is, the length of the core DNA, 147 bp, plus the linker DNA length, L. We found previously that there are two families of the two-start chromatin fiber structures characterized by different DNA topology and flexibility. (Depending on the NRL value, the energetically optimal fiber structure belongs to one of the two families.) Here we analyze the high resolution nucleosome positioning data to find whether there is any correlation between the NRL and the gene expression level in yeast. We calculate the NRL values for the two groups of genes - 25% highly expressed and 25% lowly expressed genes (out of ~3,500 yeast genes that are at least 1,000 bp long). Our results show that the average NRL=161-162 bp for the highly active genes (i.e., linker L=14-15 bp), whereas NRL=167-168 bp (i.e., linker L=20-21 bp) for the lowly transcribed genes. Based on these findings, we conclude that the highly and lowly active gene sets have distinct nucleosome fiber organization with the linker L ≈ 10n+5 and 10n, respectively. We hypothesize that organization of the most active genes in fibers with L ≈ 10n+5 (which are more flexible than the fibers with L ≈ 10n) facilitates formation of gene loops, thereby inducing transcription of these genes.

#### 2732-Pos Board B162

##### Using FRET to Monitor Nucleosome Movement by the Chd1 Chromatin Remodeler

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The chromatin remodeler, Chd1, creates regularly spaced arrays of nucleosomes. This spacing behavior correlates with a preference for shifting mononucleosomes away from DNA ends. However, the mechanism by which Chd1 senses extranucleosomal DNA is not understood. To investigate how sliding activity may be regulated, we are adopting a FRET-based nucleosome sliding assay to measure the kinetics of Chd1 directed nucleosome sliding. By labeling the end of the DNA with Cy3 and the nucleosome with Cy5, we should be able to monitor nucleosome sliding as the loss of quenching of Cy3 by Cy5 as the dye pair separates. Using a similar system, others have observed single or double exponential increases in Cy3 fluorescence corresponding to nucleosome sliding. Curiously, with Chd1 we observe an initial increasing phase followed by a pause and then a second increasing phase. In addition, after an initial drop in Cy5 fluorescence expected from FRET, a later phase shows an increase in Cy5 fluorescence above its initial level. These results suggest that not all of the fluorescence signals are reporting on DNA movement alone, and are likely being influenced by Chd1 binding to the labeled nucleosome. We are investigating other dye pairs and nucleosome constructs to isolate nucleosome sliding activity from binding or other molecular interactions that may occur during the reaction.

#### 2733-Pos Board B163

##### Development of a "Realistic" Model of the Dynamic Chromatin Fiber using an Integrated Computational Approach

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DNA in eukaryotic cells is highly compacted into a hierarchical chromatin structure to fit inside the nucleus. Linker histones play an important role in this packing. Their interaction with the nucleosomes and intervening DNA linkers, in combination with linker length, are believed to affect chromatin folding and long-range interactions. The details of chromatin structure at this level of compaction are still an open question but have profound implications