ALLOSTERIC CHANGES IN THE CENTROMERE-SPECIFIC NUCLEOSOME IN RESPONSE TO PHYSICAL FACTORS

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Abstract

Project summary

Faithful segregation of sister chromatids is critical for the transfer of genetic inheritance during cell division. This is accomplished through the physical separation of these chromatids to the opposite cell poles via the spindle apparatus. Microtubular filaments of this apparatus attach to chromatin via kinetochores which assemble via direct recognition of the epigenetic factor CENP-A, known as a centromere-specific variant of histone H3. Histones are core protein components of nucleosomes which is a disc-shaped construct that wraps around ~150 base pairs of DNA. Prior evidence indicates that open DNA termini of CENP-A containing nucleosomes are critical for mitotic fidelity. A recent cryo-EM model also supports open kinetochore configurations. Using a novel cryo-EM approach coupled with *in silico* tools of molecular modeling and high-performance computing, we demonstrated recently that the DNA termini of CENP-A nucleosomes open up asymmetrically under physiological salt conditions. In this work, we build upon these findings to show that the CENP-A nucleosome can exhibit symmetrically open ends as a result of the solvent environment and the DNA sequence. We generated extended DNA models starting from the atomistic coordinates of publicly available nucleosomes containing CENP-A (PDB ID: 6TEM) or canonical H3.2 (PDB ID: 3LZ0). We solvated these nucleosomes in a cubic water box where the salt concentration is adjusted to physiological conditions (control) or an environment rich in divalent cation (pre-mitotic). We validated that the nucleosome containing the canonical H3.2 in physiological environmental salt exhibits closed DNA termini on both sides and CENP-A containing nucleosome in physiological environmental salt exhibits one side open (the "strong" side) and the other one closed. We find that the CENP-A containing nucleosome exhibits open DNA termini in the presence of divalent ions (Fig. 1A). This behavior is more pronounced when the local DNA sequence is altered near the nucleosomal gyre known to bind DNA more strongly (Fig. 1B). In our simulations, partial unwrapping (asymmetrically open end) and unwrapping (symmetrically open ends) of CENP-A containing nucleosomes in physiological and presence of divalent ions environments show higher DNA termini fluctuation compared to H3.2 in a physiological environment (Fig. 1C). When the local DNA sequence is altered, these fluctuations become more obvious (Fig. 1D). We attribute this behavior to a CENP-A specific residue which binds DNA minor groove in a manner that is tunable by solvent electrostatics. We validate our results through delicate restriction enzyme assay measurements that mimic the early stages of kinetochore assembly wherein the pre-mitotic cell chromatin is exposed to different ions upon the disintegration of the nuclear envelope. These findings could shed light on the assembly and regulation of the early steps of the kinetochore apparatus.

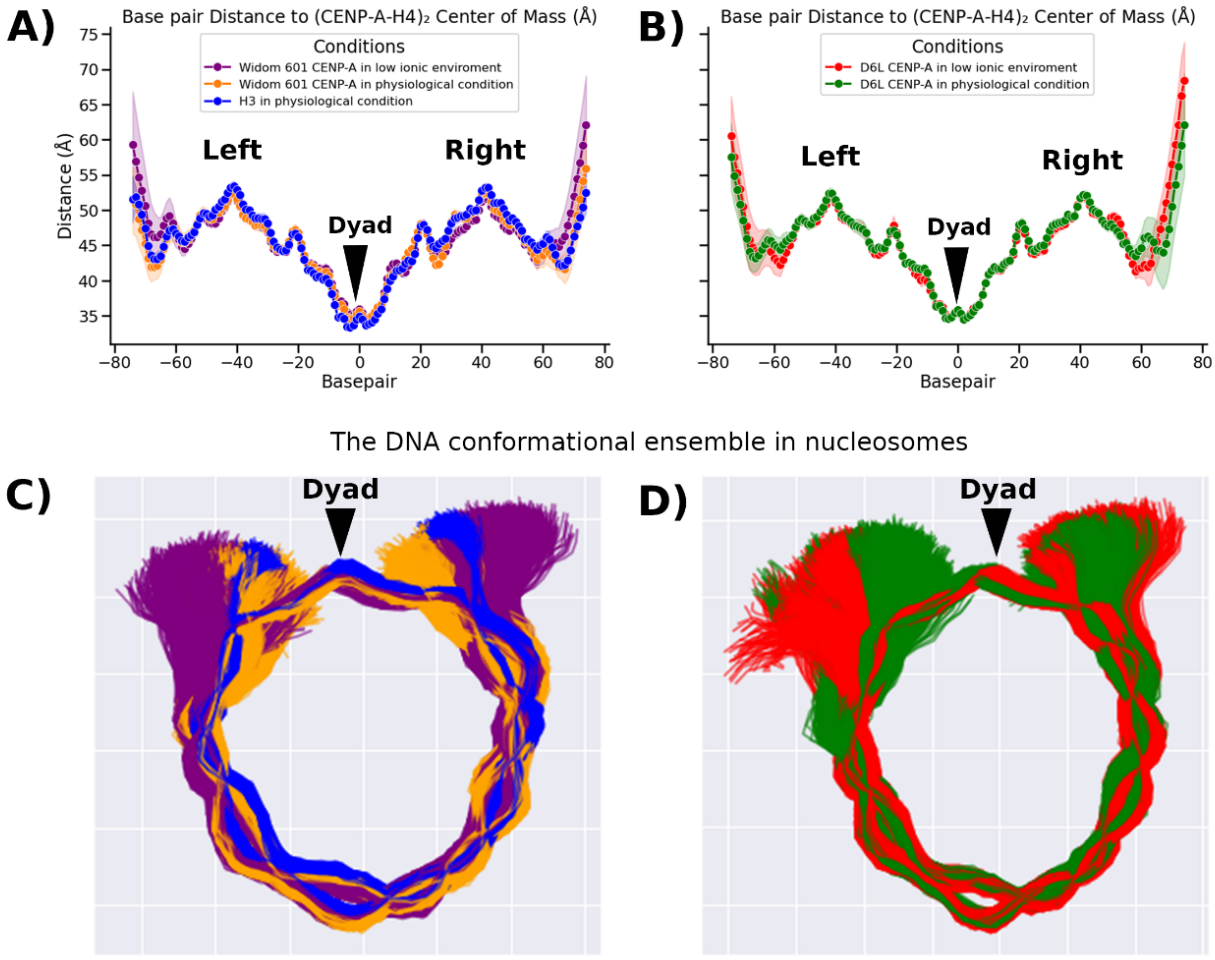

Figure 1: Solvent environment and the DNA sequence can affect CENP-A containing nucleosomal DNA termini opening. A), B) plots of the tetramer-DNA base pairs distances. A) The flexibility of H3 containing nucleosomal DNA termini (blue) is pronounced in the CENP-A containing nucleosome in the physiological environment (orange). In addition, this flexibility is more pronounced when CENP-A-containing nucleosome is put into a high divalent environment (Purple). B) Local DNA sequence alteration increases the fluctuation of DNA termini of CENP-A containing nucleosome in the physiological environment (green). This fluctuation is even more pronounced when CENP-A-containing nucleosome is put into a high divalent environment (Purple). C), D) front projections of the DNA conformational ensemble in the nucleosome.