

Dynamics of the nucleosome and chromatin fiber studied by single molecule spectroscopy and computer simulations

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We present recent studies on the structure and dynamics of mono- and oligonucleosomes, using fluorescence correlation spectroscopy and fluorescence resonance energy transfer (FRET). The effect of salt concentration, linker histone H1 and histone acetylation on the structure of mono- and trinucleosomes reconstituted on nucleosome positioning sequences was investigated by FRET in bulk solution and on single molecules, as well as scanning force microscopy (SFM) in liquid.

The distance between the linker DNA ends decreased under the effect of increasing salt concentration and also by the incorporation of H1 linker histone. The distribution of the angle formed between consecutive nucleosomes in trinucleosomes is narrowed by H1. Acetylation of all histones leads to decompaction, measured as increased distance between the DNA ends, and also increases the internucleosomal distances. Selective acetylation of histone H4, however, compacts the structure.

Single molecule spectroscopy allows us to assess the structural heterogeneity of mononucleosomes in solution. Analyzing FRET of individual nucleosomes reconstituted with nucleosome positioning sequences shows that the DNA exists in three states, two of them rigidly bound but distinct, and a third highly flexible state, which might correspond to a nucleosome repositioning intermediate.

The mechanical properties of the chromatin fiber are modeled by a Monte-Carlo model in which the DNA is approximated by a flexible segmented chain and the nucleosomes by rigid flat cylinders. This model allows for the prediction of possible higher-order structures and their mechanical properties. As examples, we show the unrolling of DNA from the histone core, the response of the 30 nm chromatin fiber to mechanical stretching and possible regimes of stable and unstable packing of chromatin.