spFRET studies of nucleosome dynamics modulated by histone modifications, histone variants and neighboring nucleosomes

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Summary

In this thesis, I describe the results of single-pair Fluorescence Resonance Energy Transfer (spFRET) studies on the dynamics of individual nucleosomes, modulated by histone modifications, histone variants, and by neighboring nucleosomes.

At the basis of the regulation of the genetic code (DNA) in eukaryotes is its organization into nucleosomes: 10 nm wide structures, in which ~ 150 basepairs (bp) of DNA are wrapped around a disk of proteins, the histone octamer. Arrays of nucleosomes are organized in fiber-like structures called chromatin. Nucleosomes modulate DNA accessibility through conformational dynamics like DNA breathing - the transient unwrapping of DNA from the nucleosome -, repositioning of nucleosomes along the DNA, or partial dissociation. Thus, nucleosomes play a crucial role in regulating all processes involving DNA, including transcription, replication and repair. Single-molecule techniques, in particular single-pair Fluorescence Resonance Energy Transfer (spFRET), have the ability to resolve such conformational dynamics in individual nucleosomes and may help to understand these processes at a fundamental level.

Chapter 1 reviews the results of experiments that have used spFRET to elucidate single-nucleosome dynamics, including fluorescence correlation spectroscopy (FCS), confocal single-molecule microscopy on freely diffusing nucleosomes and widefield total internal reflection fluorescence (TIRF) microscopy on immobilized nucleosomes. The combined spFRET studies on single nucleosomes reveal a very dynamic organization of the nucleosome, that has been shown to be modulated by post-translational modifications of the histones and by DNA sequence.

Performing spFRET experiments on nucleosomes and interpreting their results is far from trivial. Nucleosomes are susceptible to dissociation when diluted to sub-nM concentrations - typical for single-molecule experiments - and in the presence of surfaces, depending on the specific histone composition and buffer conditions. Nucleosome instability during storage and sample preparation, sample heterogeneity, and simplifications in the analysis of single-molecule fluorescence data can introduce artifacts or obscure the underlying conformational behavior of nucleosomes. **Chapter 2** describes the challenges we encountered during the preparation of nucleosome sam-

ples, the detection of spFRET with confocal fluorescence spectroscopy and the analysis of FRET efficiencies, and how we have dealt with them.

Post-translational modifications to the histone proteins play an essential role in the regulation of various processes involving DNA. Lysine acetylation of histones, for example, defines the epigenetic status of human embryonic stem cells, and orchestrates DNA replication, chromosome condensation, transcription, telomeric silencing, and DNA repair. A detailed mechanistic explanation of these phenomena is impeded by the limited availability of homogeneously acetylated histones for in vitro studies. **Chapter 3** reports a new method for the production of homogeneously and site-specifically acetylated recombinant histones by genetically encoding acetyl-lysine. Using such histones it is possible to reconstitute nucleosomes bearing defined acetylated lysine residues. With these designer nucleosomes it is demonstrated that, in contrast to the prevailing dogma, acetylation of H3 K56 does not directly affect the compaction of chromatin, and has modest effects on remodeling by SWI/SNF and RSC. However, our single-molecule FRET experiments reveal that H3 K56 acetylation increases DNA breathing 7-fold. These results provide a molecular and mechanistic insight in the cellular phenomena that have been linked to H3 K56 acetylation.

The incorporation of histone variants is another way in which eukaryotic cells regulate their DNA activity. H2A.Z is a highly conserved histone variant involved in many transcription-related functions. In Arabidopsis Thaliana, H2A.Z plays an essential role in ambient temperature sensing. The mechanism by which H2A.Z does so remains however unresolved. Both enhanced and reduced nucleosome stability have been reported. **Chapter 4** focuses on the effect of H2A.Z incorporation on nucleosome stability and dynamics. Here I show that H2A.Z-containing nucleosomes are more stable than H2A- containing nucleosomes. In single-pair FRET experiments on individual nucleosomes we found that H2A.Z-containing nucleosomes have a lower unwrapping probability and are less susceptible to dissociation during gel electrophoresis, at low concentrations, or in the presence of surfaces. Ambient temperature changes between 7 and 37 °C have no detectable effect on the dynamics of H2A.Z-containing nucleosomes. Our results suggest that H2A.Z containing nucleosomes do not directly respond to temperature changes, but that H2A.Z incorporation may act as a nucleosomal stability switch.

Finally, in **Chapter 5**, we investigate how DNA breathing is affected by extension of the linker DNA and by the presence of a neighboring nucleosome. We found that both electrostatic interactions between the entering and exiting linker DNA and nucleosome-nucleosome interactions increase unwrapping. Interactions between neighboring nucleosomes are more likely in dinucleosomes spaced by 55 bp of linker DNA than in dinucleosomes spaced by 50 bp of linker DNA. Such increased unwrapping may not only increase the accessibility of nucleosomal DNA in chromatin fibers, it may also be key to folding of nucleosomes into higher order structures.

Taken together, our data shed new light on the molecular mechanisms underlying DNA accessibility in chromatin, which in turn may play a role in the regulation of processes like transcription, replication and DNA repair. spFRET was shown to be able to reveal subtle changes in nucleosome conformation due to histone modifications, variations, or constraints imposed by linker DNA and nucleosome neighbors. The spFRET experiments described here can be extended to investigate conformational changes when embedding the nucleosome in a chromatin fiber. Moreover, single-molecule fluorescence techniques may be extended with more colors, allowing the detection of other factors that interact with nucleosomal DNA, yielding even more detailed insight in the molecular mechanisms that control our genome.