Doctoral Thesis

Nucleosome remodeling activities act on UV-damaged nucleosomes and facilitate DNA-repair

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Nucleosome Remodeling Activities Act on UV-Damaged Nucleosomes and Facilitate DNA-Repair

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Summary

UV light induces damages in DNA, leading to structural distortions that impede basic cellular functions such as transcription and replication. DNA lesions have to be repaired to prevent mutations, cancer and cell death. Cyclobutane pyrimidine dimers (CPDs) are the major DNA lesions generated by UV light. CPDs are repaired either by nucleotide excision repair (NER) or by photoreactivation. NER is a multistep pathway conserved from yeast to human. For photoreactivation, a single enzyme called photolyase uses light as energy source to repair CPDs. The mechanism of photoreversion involves flipping-out the CPD into the active site of the photolyase enzyme. In eukaryotic cells, DNA is folded in nucleosomes and higher order chromatin structures. Nucleosomes exert a repressive influence on the biological functions of DNA by restricting its accessibility to proteins. Despite of packaging in chromatin, CPDs are completely repaired by NER and by photoreactivation *in vivo*. However, preceding work demonstrated that a nucleosome positioned on a short DNA sequence (134 bps) severely inhibits photoreactivation *in vitro*. Therefore, it is not known whether *in vivo* dynamic properties of nucleosomes are sufficient for repair of nucleosomal CPDs or whether nucleosome remodeling machines might be required. Here, nucleosome reconstitution and photolyase were used to investigate how structural and dynamic properties of nucleosomes and chromatin remodeling complexes contribute to damage recognition and processing.

We reconstituted a positioned nucleosome at one end of a 226 bps long DNA sequence ('ATDED-long') to investigate whether providing space for octamer sliding facilitates nucleosomal photorepair. DNA-damage was induced by UV-light and repair was analysed by photolyase. We observed slow and inefficient repair in nucleosomal DNA, whereas DNA outside of the nucleosome was efficiently repaired. Despite of the length of the fragment, the nucleosome was neither displaced by UV damage nor during photoreactivation. Thus, the ATDED-long nucleosome mimics the *in vivo* situation, where repair by photolyase is modulated by positioned nucleosomes. In contrast to the almost complete repair of nucleosomal lesions in living cells, repair remains inhibited in the ATDED-long nucleosomes even after long incubation times, suggesting that nucleosome disruption or displacement might be required for efficient repair *in vivo*.

ATP-dependent chromatin remodeling complexes are molecular machines that use the energy of ATP hydrolysis to alter the structure of nucleosomes and promote octamer sliding. The ySWI/SNF and yISW2 remodeling complexes have been shown to play important roles in the

regulation of transcription. However, it is not known whether they also assist other DNA-dependent processes, like replication, recombination and DNA repair. In this work we found that both, ySWI/SNF and yISW2 were capable to remodel UV-damaged ATDED-long nucleosomes and facilitated repair of nucleosomal CPDs by photolyase. While ySWI/SNF altered the conformation of nucleosomal DNA and promoted more homogeneous repair, yISW2 altered the repair pattern by moving the nucleosome to a more central position on the DNA fragment. Thus, two different nucleosome remodeling complexes can act on UV-damaged nucleosomes and facilitate repair by a 'flip-out' enzyme. It is therefore possible that similar activities are engaged in living cells to relieve the inhibitory effect of nucleosomes for photoreactivation and other DNA-repair processes.