Nucleotide excision repair: from molecular mechanisms to patient phenotypes

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Introduction

Scope of the thesis

Aim and outline of the thesis

The genetic information in our cells is constantly exposed to DNA-damaging agents, resulting in the accumulation of genomic DNA lesions. If not efficiently repaired, such DNA lesions can trigger the onset of disease, including cancer. To prevent the adverse consequences of genomic DNA lesions, cells have evolved an impressive repertoire of DNA repair mechanisms that each eliminate a specific subset of DNA damage from the genome. One of these DNA repair pathways is nucleotide excision repair (NER), which removes a wide variety of structurally unrelated bulky lesions from the human genome. While extensive research during the last decades has taught us a lot about these DNA repair pathways, our understanding of the precisely orchestrated molecular mechanisms underlying DNA damage repair in a chromatin environment is far from complete.

This thesis aims to gain a better understanding of NER, to elucidate new molecular mechanisms and proteins that orchestrate how DNA repair is carried out on genomic DNA that is tightly packed in chromatin inside the living cell. It is important to obtain a better clinical picture of how inherited defects in DNA repair genes shapes phenotypes in patients with DNA repair-deficiency disorders.

In **chapter 1**, we review the role of post-translational modifications (PTMs) in NER during damage recognition, particularly in the NER sub-pathway global genome repair (GGR). In GGR the two core proteins XPC and DDB2 enable damage recognition. When DNA lesions are present in chromatinized genomic DNA, which is less accessible for the repair proteins, there are several mechanisms to improve access for the repair proteins. PTMs occur in histone proteins and on repair proteins themselves, enabling the lesion to become more accessible for repair proteins. We describe the role of several PTMs, including ubiquitylation, SUMOylation, methylation, poly(ADP-ribos)ylation and acetylation during DNA repair. In addition to PTMs, chromatin remodelers are also involved in this process. They open up chromatin in an energy-consuming reaction, thereby stimulating NER. This review chapter thus highlights the complexity of DNA repair in chromatin.

In **chapter 2**, we provide evidence for the importance of PARylation to recruit a chromatin remodeler during GGR. We identify the poly-(ADP-ribose) polymerases PARP1 and PARP2 as constitutive interactors of XPC. The biochemical interaction between these proteins results in the PARylation of XPC at UV lesions and the XPC-dependent stimulation of

the poly-(ADP-ribose) response. This enables the recruitment of the poly-(ADP-ribose)-regulated chromatin remodeler ALC1. Notably, we found that both PARP2 and ALC1 are required for the efficient clearing of CPD lesions.

In **chapter 3**, we studied the role of the chromatin modulator HMGN1 in the human NER sub-pathway transcription-coupled repair. Previously, studies in mice have revealed that HMGN1, is required to enhance the repair of UV-induced lesions in transcribed genes. To our surprise, we showed that neither HMGN1 nor the related HMGN2 is required for human TCR. The functional difference between mice and humans might be partially explained by species-specific genetic differences.

In **chapter 4,** we studied the landscape of proteins that either associates with or dissociates from UV-irradiated chromatin by employing chromatin mass spectrometry (CHROMASS) in *Xenopus laevis* (African clawed frog) egg extracts. Through this method, we observed that HMGN and HMGA proteins dissociate while HMGB showed a strong association with UV-irradiated chromatin in both frog egg extracts and cultured human cells. Future studies will be needed to reveal the functional relevance of these UV-induced chromatin interactions.

In **chapter 5**, we analysed the impact of a rare mutation in one of the NER endonucleases at the molecular level to better understand the phenotype observed in patients. We found that bi-allelic ERCC1 mutations in two patients impede DNA damage repair and cause liver and kidney dysfunction. Genomic sequencing identified a deletion and a missense variant (R156W) within ERCC1 that disrupts a salt bridge below the XPA-binding pocket. Altogether, the R156W mutation in ERCC1 has a severe effect on NER and a considerable impact on interstrand crosslink repair, together resulting in a unique phenotype combining short stature, photosensitivity and progressive liver and kidney dysfunction.

In **chapter 6** the scientific findings of this thesis are discussed in a broader context. Here, we provide a basis for further discussions and future perspectives. By highlighting our research findings and new insights into the molecular mechanisms of NER, we aim to push forward future research in the field.