Nucleotide excision repair : a multi-step mechanism required to maintain genome integrity

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INTRODUCTION AND OUTLINE OF THE THESIS

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GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

DNA carries the genetic information required for sustained life and reproduction. Hence, DNA damage induced by continuous exposure to both endogenous and exogenous genotoxic agents can have serious consequences for all organisms. Nucleotide excision repair (NER) is a multi-step enzymatic process which recognizes and eliminates a wide spectrum of lesions causing large distortions of the DNA structure such as UV induced lesions and bulky chemical adducts (Pfeifer, 1997; Wogan et al., 2004). The consequences of defective NER are apparent from the clinical symptoms of individuals affected by three disorders associated with reduced NER capacities- Xeroderma Pigmentosum (XP), Cockayne Syndrome (CS) and Trichothiodystrophy (TTD). These disorders are linked by increased sensitivity to UV-irradiation and patients have a greatly elevated cancer incidence (XP) and multi-system defects which can include immunological and neurological disorders (Mitchell et al., 2003).

The aim of the work outlined in this thesis was to identify novel factors and gain new insights into the global genome repair pathway in human cells. Although most core factors are known we are only beginning to understand the complex organization of this repair process and regulation of the various NER factors. In **chapter 2**, we focus on the importance of genome stability and the various cellular defense mechanisms as a response to various types of DNA damage. In addition, the various DNA repair pathways involved in the repair of various types of DNA damage (such as single- and double strand breaks, oxidative lesions as well as UV-induced damage) are briefly described. **Chapter 3** provides a more detailed explanation of NER. In humans, NER is solely responsible for the repair of UV-induced lesions. The consequences of defective NER, as well as the 2 sub-pathways of NER, Transcription-coupled repair (TC-NER) and Global genome repair (GG-NER) are therefore described in detail. In addition we present a model for GG-NER (in mammals) describing the probable sequence of events. This model is based on data from previously published work, as well as on experimental evidence described in this thesis. Genetic information is packaged with histones and other accessory proteins into chromatin. Chromatin remodeling is essential for key cellular processes such as the regulation of gene expression, DNA replication and DNA repair in order to alter the accessibility of DNA. In addition to ATP-dependent chromatin modifications (by chromatin remodeling complexes) numerous covalent modifications of core histones have been identified which include acetylation, sumoylation, methylation, phosphorylation and ubiquitination. These chromatin alterations are described as the "histone code". The importance of chromatin remodeling mechanisms and chromatin modifications is described in **chapter 4**.

The role of damage recognition factor UV-DDB in NER is described in **chapter 5**. UV-DDB was shown to form a stable complex when bound to 6-4PP which allowed subsequent NER proteins such as XPC-hHR23B to accumulate and verify the lesion resulting in efficient 6-4PP repair. In **chapter 6**, the *in vivo* properties of DDB2, Cul4A and XPC are described. The properties of fluorescently labeled DDB2 in wild-type and XPC-deficient cells were analyzed and results suggest that the bulk of DDB2 interacts with lesions independently of XPC. We propose that DDB2 prepares UV-damaged chromatin for the assembly of

NER complexes. Turning our attention to the later stages of NER, the involvement of DNA polymerases and DNA ligases are described in **chapter 7**. Here we show for the first time that DNA Ligase III and its binding partner XRCC1 are involved in the sealing of chromosomal nicks introduced by NER. In fact, 2 distinct ligase-polymerase complexes are functional dependent on the cell-cycle status. **Chapter 8** focuses on the stability of the NER complex and the assembly and disassembly of the various NER components at UV-induced lesions. Here we show that all pre-incision NER proteins with the exception of RPA are able to disassemble from NER complexes and associate with unprocessed sites of damage. In contrast, post-incision complexes together with RPA remain associated with the incomplete NER synthesis sites. We propose a model wherein RPA plays a pivotal role in preventing new incision events when gap filling/sealing is not completed or is disturbed thereby averting further generation of DNA strand breaks that could lead to mutagenic and recombinogenic events with deleterious consequences for cells and organisms. It is known that arsenic acts as a co-carcinogen by inhibiting the repair of UV-induced lesions by NER but the exact mechanism has not been described until now. In **chapter 9** we show that the late stages of NER are affected and in particular we show a specific inhibitory affect of arsenic on DNA ligase III function.