Universiteit
Leiden
The Netherlands

Nucleotide excision repair : complexes and complexities : a study of global genome repair in human cells

Volker, Marcel

Citation
Volker, M. (2006, May 15). *Nucleotide excision repair : complexes and complexities : a study of global genome repair in human cells*. Retrieved from https://hdl.handle.net/1887/4390

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/4390

Note: To cite this publication please use the final published version (if applicable).

Nucleotide excision repair – complexes and complexities

A study of global genome repair in human cells

Nucleotide excision repair – complexes and complexities

A study of global genome repair in human cells

proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus Dr. D. D. Breimer,
hoogleraar aan de faculteit der Wiskunde en
Natuurwetenschappen en die der Geneeskunde,
volgens besluit van het College voor Promoties
te verdedigen op maandag 15 mei 2006
klokke 16.15 uur

door

Marcel Volker

Geboren te Leiderdorp,
in 1976

Promotiecommissie

Promotores	Prof. Dr. L.H.F. Mullenders Prof. Dr. Ir. A.A. van Zeeland
Referent	Dr. W. Vermeulen (Erasmus Universiteit Rotterdam)
Overige leden	Prof. Dr. H. van Steeg Prof. Dr. H. Tanke

All we have to decide is what to do with the time that is given us

J.R.R. Tolkien - the Lord of the Rings

aan mijn vader

voor mijn moeder

Contents

Aim and outline of the thesis

Aim and outline of the thesis

Of all exogenous agents that damage genomic DNA and hence present a threat to the integrity of its genetic information, the ultraviolet B (UVB) component of sunlight possesses high clinical relevance because of its abundance. UVB induces predominantly two types of photolesions: cyclobutane pyrimidine dimers (CPD) and pyrimidine 6-4 pyrimidone photoproducts (6-4 photoproducts or 6-4PP). Uniquely, placental mammals rely solely on the nucleotide excision repair (NER) system to repair these photolesions. In addition, NER is capable of removing a wide range of bulky, helix-distorting lesions, including numerous chemical adducts such as those caused by the anticancer drug *cis*-diamminedichloroplatinum(II) (cisplatin), the poison gas nitrogen mustard and polycyclic aromatic hydrocarbons found in burnt food and cigarette smoke. The relevance of the NER pathway, particularly in the removal of UV-induced photolesions, is emphasised by the existence of three inherited disorders caused by mutations in genes encoding NER proteins. Patients suffering from these disorders invariably display hypersensitivity to sunlight, and patients suffering from one disease, xeroderma pigmentosum, combine this with a dramatically increased risk of developing skin cancers.

In the first part of this thesis, the different cellular defence mechanisms against various types of DNA damage are described. Chapter 1 summarises the possible reactions of a cell to DNA damage and briefly sketches the DNA repair pathways. Chapters 2 and 3 concern themselves solely with NER: chapter 2 introduces some background important to understanding NER while chapter 3 provides a detailed step-by-step description of the global genome repair pathway, highlighting the roles of every factor involved. Subsequently chapter 4 elaborates on the inhibition of transcription that is observed after the introduction of NER-types of DNA damage. Chapter 5 puts NER in a nuclear perspective, describing its interplay with chromatin, the natural substrate of any DNA repair pathway.

The research presented in this thesis is focused on various aspects of the NER pathway in live cells. One of the main questions addressed is whether the NER complex assembles on the lesion in a sequential manner or whether it pre-exists as a so-called 'repairosome'. Experimental evidence as described in chapter 6 suggests that a sequential assembly of NER proteins on DNA lesions is most likely. The technique of local UV irradiation used in this study was utilised to address another question: the possible involvement of transcription factor TFIIH in the UV-induced temporary inhibition of transcription. The result of this research, together with a detailed description of the local irradiation method, is presented in chapter 7. The cellular characteristics of XPA, an NER protein involved in damage verification were further investigated in chapter 8. In contrast to what had been reported previously, we found that XPA does not form a cellular complex with the single-strand binding protein RPA. After having investigated the functioning of essential NER proteins, the role of the accessory damage recognition factor UV-DDB in NER was assessed. Using a significantly lower UV dose compared to that used in earlier published research, we discovered a previously ill-recognised role for UV-DDB in 6-4PP repair which is described in chapter 9. Turning the attention to the later stages of the NER reaction, the involvement of DNA polymerases δ and ε and DNA ligase I in the DNA resynthesis step of NER *in vivo* was investigated, while concurrently the stability of the preincision NER complex and the DNA resynthesis complex were assessed. The results of this research are presented in chapter 10.