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

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Summary

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DNA, the physical carrier of our genetic information, is not as inert as one imagines or as one might want it to be. It deteriorates spontaneously, and it is also constantly threatened by both exogenous and endogenous chemical and physical agents. If the cell does not deal appropriately with damage to its DNA, the consequences may be serious. One possible effect is the stalling of DNA and RNA polymerases on the lesions. A stalled polymerase physically prevents a cell from replicating its DNA, and hence from dividing; this could lead to the cell's death. Another possibility is that the wrong nucleotide is inserted opposite the lesion, introducing mutations that can cause malfunctioning proteins and eventually could result in cancer.

Several systems have evolved that counteract the harmful effects of damaged DNA. Generally, the cell will stop its cell cycle, to prevent the replicative polymerases from attempting to copy the damaged DNA. At the same time, this gives the cell time to try and repair the damage, using its DNA repair pathways. Sometimes, the DNA may be damaged to such an extent that the cell assesses repair to be impossible; in such cases, the cell may commit to the 'ordered selfdisassembly program' known as apoptosis. By undergoing apoptosis and removing itself, the cell prevents more serious damage (e.g. the formation of cancer) to the entire organism. **Chapter 1** briefly describes these responses with a focus on the DNA repair pathways, outlining their mechanism of action and the main factors involved.

The research in this thesis is focused on the DNA repair system nucleotide excision repair (NER). The NER pathway is capable of repairing a wide variety of DNA lesions, including photolesions caused by the ultraviolet component of sunlight (cyclobutane pyrimidine dimers, CPD and pyrimidone 6-4 pyrimidone photoproducts, 6-4PP), small DNA adducts caused by the anticancer drug cisplatin, and large adducts introduced by the aromatic carbohydrates found in burnt food and cigarette smoke. At the molecular level, the mechanism of NER consists of several steps: recognition of the damaged DNA; unwinding of the DNA double helix immediately surrounding the lesion; and incision of the DNA strand containing the damage on both sides of the lesion, after which the oligonucleotide containing the damage is removed. Then, using the undamaged opposite strand of DNA as a template the gap is filled and subsequently sealed, resulting in fully restored DNA.

NER has been extensively researched; **chapter 2** provides an overview of the basic concepts of NER, including a brief sketch of one of the two subpathways of which it is composed, i.e. transcription-coupled repair (TCR). TCR couples repair of DNA lesions to RNA polymerases stalled on damaged DNA, allowing the lesions to be removed rapidly so that they obstruct transcription as short as possible. The second pathway of NER is global genome repair (GGR) which indiscriminately removes lesions from the entire genome; it is elaborated on in chapter 3. Also discussed in chapter 2 are the consequences of defective NER as evident from several inherited disorders. People with a defect in GGR suffer from the disorder called xeroderma pigmentosum (XP). XP patients display several features resulting from their repair defect, but the most striking (and most fundamental) of all is an extremely increased risk of developing skin cancers at sun-exposed parts of their skin, underlining the importance of GGR in preventing cancer. On the other hand, people that suffer from Cockayne syndrome (CS) have a defect in TCR; in contrast to XP, CS does not comprise cancer-proneness but instead CS patients display (amongst other features) growth defects and mental retardation. These features are ultimately linked to the defect in transcription that is described in detail in chapter 4. Finally chapter 2

describes two basic concepts upon which our current thinking of NER is based: the idea that the proteins involved in NER are recruited to the lesion sequentially (as opposed to all at once), and the 'bipartite' model for damage recognition that proposes that NER does not recognise a DNA lesion in a single step but in two, each step sensing a different characteristic of the damaged DNA: disturbing of the base stacking in the DNA helix and the presence of a chemical alteration in the DNA.

As mentioned, **chapter 3** then provides a detailed account of the major NER pathway, i.e. GGR. The proteins involved, their respective roles and their interactions are extensively discussed. First, the two so-called 'damage recognition factors', UV-DDB and XPC-hHR23B are covered. While XPC-hHR23B is generally considered the factor necessary to initiate GGR by detecting damaged DNA, UV-DDB may play an assisting role in this recognition step. UV-DDB and its role in repair of 6-4 photoproducts is elaborated on in chapter 9. The next factor to be addressed is the multifunctional, multisubunit TFIIH. Initially thought to be merely necessary to open up the DNA to provide access to subsequent proteins, it has become clear that in fact, TFIIH plays a very active role in regulation of these proteins and perhaps also assists in recognition of the damage. Then, XPA is covered. At one time proposed to be the damage recognition protein (assisted by RPA), it is now clear XPA is involved in the second step in the bipartite NER model: verification of the presence of a lesion in DNA. A model is presented in which XPA accomplishes this through the tight regulation of the subsequent dual incision steps; XPA might cooperate closely with TFIIH in this phase of NER. As mentioned, RPA was once thought to be involved in damage recognition together with XPA. However, it has been established that RPA, due to its extraordinary high affinity for single-strand DNA, plays an important role in NER in stabilising the undamaged strand when the helix has been opened up and the damaged piece of DNA has been removed. Additionally, RPA is of importance for the regulation of the two endonucleases XPG and ERCC1-XPF that make the nicks to remove the damaged DNA. The order of binding to the NER complex of XPA, RPA and XPG is not clear. Evidence for and against several binding orders is discussed, as well as the proposal that in fact, these proteins may not bind in a specific but in a random order. ERCC1-XPF is the last factor to be incorporated into the preincision NER complex before dual incision occurs. By the time the oligonucleotide containing the damage is removed, all preincision NER proteins are removed from the DNA apart from RPA and possibly XPG (see also chapter 10); unclarity still exists whether some proteins may leave the complex earlier. Especially XPC-hHR23B has in a number of reports been found to leave the NER complex around the same time that XPG enters it. To the single-strand gap (with RPA bound to it), proteins are then recruited that are also involved in DNA replication: the ring-shaped clamp for DNA polymerases, PCNA; RF-C, which loads PCNA onto the DNA; the DNA polymerases delta and/or epsilon, and the DNA ligase I.

Transcription interacts with NER in one more way that is not discussed in chapters 1 through 3. Upon the infliction of DNA damages that are targeted by TCR (including the UV-induced photolesions), transcription is reduced; normally this inhibition is released when the lesions are removed. Strikingly, also transcription of genes that are not damaged is reduced. As yet it is unknown what causes this effect. **Chapter 4** discusses possible mechanisms for both the origin of the inhibition and how undamaged genes may be affected. Fundamental to the inhibition is the hyperphosphorylation of RNA polymerase II, resulting in its inactivation in transcription initiation. In CS cells this hyperphosphorylation is not reversed even when damages have been removed, and the resulting permanent reduction in transcription is thought to be the root cause of many of the CS patients' characteristics.

The final introductory chapter, **chapter 5**, puts NER in the context of the nucleus, where it has to deal with DNA being packaged into chromatin, which in itself has an inhibitory effect on NER. Chromatin remodelling can however alleviate much of this inhibition, both by 'shuffling' histones around on the DNA and by modifying the histones so that the DNA is less efficiently packed by them. Proteins involved in either mechanism that have been found to enhance NER, as well as proteins that in some other way reduce the restrictive effects of chromatin on NER are discussed in this chapter. Finally, also the post-NER chromatin restoration is highlighted.

Most of the research into NER has been performed *in vitro*, i.e. using cell extracts and/or purified proteins. The research described in this thesis, on the other hand, was mostly done in intact cells. The major advantage of using intact cells is that there are no variable external circumstances (e.g. salt concentrations, activity of purified proteins) that can influence the outcome of experiments.

In **chapter 6**, the order of binding of several NER proteins to the NER complex is described. To be able to study this, we developed a method to study NER and other processes interacting with UV-induced DNA damage. In this elegant and simple method, cells grown on coverslips are covered with a polycarbonate filter with pores that leave a small area of the nucleus uncovered. Upon UV irradiation, the polycarbonate blocks all UV so that cell nuclei are only exposed to UV directly underneath the pores (this method is therefore usually referred to as 'local UV irradiation'; it is schematically depicted in chapter 8, figure 5). Processes that react to UV lesions can be visualised in these spots using for instance indirect immunofluorescence. We firstly found that XPC is the first core NER protein to bind to UV-lesions and thus, to be the damage recognition protein in GGR. XPA, the other candidate for this role, was shown to bind later, after the recruitment of TFIIH. These findings corroborated in vivo what had been reported by others in vitro. Secondly, XPA and XPG were found not to depend upon one another for their recruitment to the NER complex and finally, the recruitment of ERCC1-XPF was found to depend on XPA, but not XPG.

Chapter 7 describes the method of 'local irradiation' in greater detail. Furthermore, it was used to study the relation between UV irradiation and the abovementioned transcription inhibition. We found that the inhibition was restricted to areas of the nucleus that are UV-irradiated while in the remainder of the nucleus, transcription carried on as normal. We concluded that the signal for transcription inhibition is not propagated by a factor that can freely diffuse through the nucleus, such as TFIIH (which was a candidate factor).

A technique that is being used extensively in the research in live cells is the coupling of a protein species of interest to green fluorescent protein (GFP). This enables one to follow the proteins in the living cell using a confocal microscope. In **chapter 8**, the behaviour of GFP-tagged XPA is described. Measuring its rate of diffusion enabled us to determine that XPA was not travelling through the nucleus as part of a large protein complex such as a 'repairosome' (i.e., a large stable complex containing most repair factors), providing more evidence for the sequential assembly model described in chapter 2. We also found that after local UV irradiation RPA was incorporated into the NER complex even in the absence of XPA, overturning a number of older reports claiming that a complex of XPA and RPA was functional in binding to DNA lesions.

The UV-DDB protein appears only to be required for the repair of the UV-induced CPD, while repair of the other major type of UV-induced photolesions, the 6-4PP seemed hardly or not affected in cells lacking UV-DDB. This was especially puzzling as UV-DDB binds with more affinity to the latter type of lesion than the former. Using a variety of methods, in **chapter 9** we

showed that UV-DDB could in fact have a large impact on the repair of 6-4PP. However because of the peculiar characteristic of UV-DDB to be degraded during repair its effect was limited to a small number of lesions. If a small number of 6-4PP was introduced, their repair was greatly accelerated by the presence of UV-DDB; if however a high UV dose was used, this effect was lost. In that case, repair of 6-4PP was carried out independently of UV-DDB, because in contrast to CPD, 6-4PP can be recognised by XPC and subsequently repaired.

In vitro research has suggested that DNA polymerases delta and epsilon, as well as DNA ligase I, are involved in the final stages of NER: DNA resynthesis and ligation. In **chapter 10**, using local UV irradiation and immunofluorescence, we found strong evidence that this is also the case *in vivo*, as both DNA polymerases delta and epsilon and DNA ligase I are found to accumulate at the sites of UV damage. Furthermore, irradiating cells twice – once globally and once locally – we were able to determine whether NER proteins leave the complex, and at what stage of the reaction. We found that all preincision factors except RPA dissociate following dual incision; subsequently the resynthesis and ligation proteins were recruited. In the absence of dual incision the preincision complex was stable, and no recruitment of DNA polymerases or DNA ligase could be observed.