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

Volker, Marcel

Chapter 1

Cellular responses to DNA damage

1 Cellular responses to DNA damage

Despite the fact that the information stored in the genome is very stable from generation to generation, the physical carrier of this information, the DNA itself, is not completely inert at the same timescale. Rather, it continually deteriorates spontaneously and is exposed to numerous endogenous and exogenous agents that damage it. These alterations to the DNA directly threaten cellular survival by interfering with processes such as replication and transcription. Indirectly the cell is endangered when damages lead to mutations or chromosomal aberrations, which in turn can lead to cellular malfunctioning or worse, cell death. In higher multicellular organisms like humans, the formation of cancer is a particular outcome of cellular malfunctioning that threatens survival not of the cell, but of the entire organism. Cells respond to the infliction of damage to their DNA by activating a DNA damage signalling cascade and inducing various DNA repair mechanisms.

1.1 Cell cycle arrest

When DNA damage is present at various checkpoints during the cell cycle (the G1/S checkpoint at the entry of S-phase and the G2/M checkpoint at the transition from G2- to M-phase) this will result in a cell cycle arrest. The G1/S-phase arrest is especially important as this arrest prevents the cell from attempting to replicate damaged DNA. Such an attempt would not only risk the introduction of mutations but moreover the stalling of the replicative machinery on DNA lesions or even the abortion of replication in an attempt to replicate across a double-strand DNA break. The latter event is especially deleterious and is an important source of genomic instability, giving rise to chromosomal aberrations such as loss of heterozygosity. Arresting its cell cycle not only prevents the cell from this major harm, it also gives the opportunity for 'damage assessment'. If the DNA is deemed to be damaged beyond repair, a cell may commit to apoptosis, the process of regulated cell death. On the other hand, the cell possesses a variety of DNA repair systems that are capable of repairing many different types of DNA damages. If and when repair has been completed, the cell may resume its cell cycle. The following chapter briefly describes the various mechanisms that exist to repair damaged DNA.

1.2 DNA repair mechanisms

Direct reversal

The easiest way to deal with DNA lesions is to convert the modified nucleotides back to their original structure without even removing them from the sugar-phosphate backbone. Two major repair systems use this so-called direct reversal mechanism.

First, photoreactivation mediated by photolyases restores the UV-induced CPD or 6-4PP to their original pyrimidines using energy from absorbed light. The photolyase uses a first chromophore as light-harvesting antenna to capture a photon of appropriate wavelength. The resulting excitation energy is transmitted to a second chromophore positioned near the photolesion, and the photolesion is subsequently split into its original two nucleotides. Typically, photolyases are specific for a single type of photolesion, targeting either CPD or 6-4PP but not both. Photolyases

are found in a range of species from bacteria and lower eukaryotes to plants and many animals including marsupials, but placental mammals are curiously devoid of this system.

The second example of direct reversal is the enzyme methylguanine DNA methyltransferase (MGMT) which is present in virtually all species throughout all kingdoms. It restores O^6-methylguanine back to guanine by transferring the methyl group onto a cysteine in its active site. The formed covalent methyl-cysteine bond is stable, inactivating the enzyme after only one round of repair; hence, MGMT is referred to as a suicide enzyme.

Base excision repair and single-strand break repair

Base excision repair (BER) deals with relatively mild DNA helix-distorting lesions that for a large part are produced by the cell's mitochondrial respiration processes, resulting in lesions such as 7,8-dihydro-8-oxoguanine (8-oxoguanine) and 5,6-dihydro-5,6-dihydroxythymine (thymine glycol). Other endogenous lesions tackled by BER include methylated bases, apurinic/apyrimidinic (AP)-sites formed after the spontaneous cleavage of a sugar-base bond, and single-strand breaks formed after a cleavage of the sugar-phosphate chain. Finally also uracil, which is introduced in DNA by the spontaneous deamination of methylated cytosine, is targeted by BER. These lesions are formed at high rates; estimates of the number of lesions that BER processes daily in a human cell range from ten thousand for oxidative damages only (Ames et al., 1993) to one million in total (Holmquist, 1998). To recognise the various base damages, BER employs an array of damage-specific glycosylases, including enzymes that recognise oxidised pyrimidines (8-oxoguanine glycosylase, thymine glycol glycosylase) and alkylated purines (methyl-purine glycosylase). Uracil is recognised specifically by uracil-DNA glycosylase.

Depending on the nature of the lesion and the glycosylase involved, BER can proceed via a number of routes. Firstly, the lesion may be processed by a glycosylase with 3'-β-lyase activity (a bifunctional glycosylase). This type of glycosylase cleaves off the damaged base and subsequently also incises the sugar-phosphate backbone 3' to the resulting AP site. 3' trimming by pol β or the HAP1 enzyme removes the remaining sugar residue; pol β then inserts the correct nucleotide followed by recruitment of XRCC1 and DNA ligase III, the latter of which ligates the nick. As the repair patch is only 1 nt long this pathway is referred to as short-patch BER. Secondly, when the glycosylase has no associated 3'-β-lyase activity (a monofunctional glycosylase), after the creation of the AP-site, the sugar-phosphate backbone is cleaved by HAP1 or pol β on the 5' side of the sugar. From here, BER can proceed via a short-patch or a long-patch repair pathway, depending on the nature of the damage. An unmodified AP-site will result in short-patch repair: pol β inserts 1 nt while displacing the sugar, followed by recruitment of XRCC1 and DNA ligase III and ligation of the nick. A reduced or oxidised AP-site however will lead to long-patch repair, a process involving pol β, pol δ or pol ε in combination with the replication factors RF-C and PCNA (and possibly RPA), where a patch of 2-10 nt is displaced while new nt are inserted. The resulting single-strand 'flap' is cleaved off by FEN1, after which the nick is sealed by DNA ligase I. For an extensive review of BER, the reader is referred to Barnes and Lindahl (2004).

A similar mechanism is employed in repair of single-strand breaks. These are recognised, bound and protected from recombination by the poly(ADP-ribose)polymerase 1 (PARP1) protein. After PARP1 has bound possible 'dirty' ends are trimmed off and XRCC1 is recruited. This protein then recruits proteins such as DNA polymerase β and DNA ligase III, and repair is completed via the latter stages of the BER pathway.

Double-strand break repair

Among the most cytotoxic DNA lesions are double-strand breaks (DSB), which can be formed either exogenously by high-energy radiation, or endogenously during mitotic recombination or during V(D)J recombination. As little as one DSB can cause cell death during mitosis; it is therefore of vital importance to the cell to repair any DSB that might arise. To quickly eliminate these lesions different DSB repair (DSBR) mechanisms are operational; i.e. non-homologous end joining (NHEJ) and homologous recombination (HR).

In the process of NHEJ, the ends of the broken strands are bound by the Ku70/Ku80 heterodimer, which attracts DNA-PKcs. In addition, a role for the Rad50-Mre11-Nbs1 protein complex in NHEJ was suggested but is as yet controversial, i.e. to bring the damaged ends together (de Jager et al., 2001). A defect in the Artemis protein gives rise to defective NHEJ and genomic instability (Moshous et al., 2001; Rooney et al., 2003) and Artemis is therefore thought to be involved in trimming of termini that require processing, i.e. break ends containing base damages (or hairpins in V(D)J recombination) (Jeggo and O'Neill, 2002; Riballo et al., 2004), before the break ends are rejoined by DNA-PKcs. Finally, the ends are ligated by the DNA ligase IV-XRCC4 heterodimer. Any processing of breaks prior to ligation occurs at the expense of losing genetic information (a few nucleotides); consequently the process of NHEJ can be considered as an error prone DSB repair process.

HR is a complex reaction that uses the intact information on the sister chromatid or the sister chromosome to restore information lost in the break. Firstly, the ends of the break are bound by Rad52 protein, possibly following end trimming by the Rad50-Mre11-Nbs1 (RMN) complex. This process gives rise to single-strand regions with 3' overhangs, on which Rad51 forms nucleoprotein filaments that search for homologous duplex DNA. Here, the RMN complex may also function in bringing an end together with an intact sister chromatid (de Jager et al., 2001). Once regions of homology have been found, the DNA strands are exchanged in a process that requires the Rad52, Rad54 and Rad55/57 proteins as well as the single-strand binding protein RPA. A DNA synthesis process followed by ligation restores the missing information and finally the crossed DNA strands are resolved to yield two DNA duplexes.

Finally, single-strand annealing (SSA) is a process that trims off the ends of a DSB with an exonuclease – possibly the RMN complex – until homologous regions are exposed on both sides of the break. These are then paired and the ssDNA overhangs cleaved off so that the ends can be ligated. RPA and Rad52 are likely candidates to participate in SSA. SSA can be considered as a subpathway of HR.

Unicellular organisms and mammalian stem and germ cells require their genetic information to remain intact, and therefore tend to rely more on the error-free HR pathway to repair DSB. On the other hand the less complex but error-prone NHEJ can be utilised without great risk by differentiated somatic cells. Even so, the use of HR is usually restricted to S-phase, when the sister chromatid is nearby to be used as a template. Outside S-phase, misalignment of repetitive DNA sequences might lead to deletions or translocations and hence the use of NHEJ tends to prevail.

Nucleotide excision repair

Nucleotide excision repair (NER) is the most versatile repair pathway because of its broad substrate specificity. NER is responsible for the removal of bulky, helix-distorting lesions from DNA. These lesions can result from a wide variety of agents, including the UV component of sunlight (introducing CPD and 6-4PP), benzo[a]pyrene and N-acetoxy-2-acetylaminofluorene (NA-AAF) (giving rise to

bulky DNA adducts), and cisplatin (leading to intrastrand crosslinks). The basic mechanism of NER is evolutionarily highly conserved and is found in many species, from bacteria to mammals with even significant structural homology between yeast and mammalian NER factors.

The first stage of NER, up to and including dual incision, requires six factors and several consecutive steps can be discerned. First, the damage is recognised by the XPC-hHR23B heterodimer. The helix around the lesion is then opened by transcription initiation factor IIH (TFIIH) and possibly RPA, after which the damage is verified (or demarcated) by XPA. This is followed by dual incision by two structure-specific endonucleases, XPG and the ERCC1-XPF heterodimer, which incise the damaged strand on each side of the lesion. The resulting damage-containing oligomer (in humans of a typical length of 24-30 nucleotides) is then released. In the second (postincision) stage of NER resynthesis across the resulting gap occurs which requires several proteins: RPA; PCNA, the processivity factor for replicative DNA polymerases; RF-C to load PCNA onto the DNA; and either DNA pol δ or pol ε. Finally, DNA ligase I ligates the nick. Since the research in this thesis focuses on NER, the subsequent chapters describe NER in far greater detail.

Replication-associated responses

Postreplication repair

Despite the presence of various repair mechanisms, DNA lesions occasionally escape repair and persist into S-phase. Also when a cell is subject to DNA damage during S-phase, its DNA replication machinery will encounter DNA lesions, many types of which form a potent block for the replicative polymerases α, δ and ε. These polymerases possess high fidelity and processivity to faithfully duplicate the genome during S-phase, but are incapable of coping with damaged nucleotides, causing the replication fork to stall. This can have disastrous consequences, such as the introduction of double-strand breaks (see above). Moreover, replication blocks form a potent signal to induce apoptosis. To avoid the deleterious effects of blocked replication, cells utilise several damage tolerance pathways, which allow the disturbed replicative polymerases to bypass blocking lesions. The two main routes of this so-called postreplication repair (PRR) pathway are translesion synthesis (TLS) and damage avoidance. For recent reviews on this subject, see Friedberg (2005) and Prakash et al. (2005).

Translesion synthesis

During translesion synthesis, the replicative polymerase is temporarily replaced with a polymerase capable of synthesising across the lesion and extending from the resulting mispaired bases. The last few years a range of DNA polymerases involved in TLS has been discovered; among them DNA polymerases η, ι, κ and ζ, and Rev1, each displaying different TLS capabilities and fidelities (Prakash et al., 2005). Generally, TLS polymerases possess a lower fidelity on undamaged DNA than the dedicated replication polymerases, most likely as a direct consequence of their wider active site pocket, needed to accommodate non-Watson-Crick base pairs during the replication past DNA lesions. As a consequence, TLS polymerases have a higher chance of inserting the wrong nucleotide opposite an undamaged template. Similarly many TLS polymerases do not incorporate the correct nucleotide opposite a damaged template; thus TLS is generally considered an error-prone process, leading to an increase in mutation rate. One notable exception to this latter 'rule' is the insertion of the correct nucleotides, i.e. two adenines, opposite a thymine dimer by DNA pol η.

Damage avoidance: template switching and daughter-strand gap repair

In contrast to TLS, which is usually error-prone, two other damage tolerance systems exist that are essentially error-free. During template switching, the DNA polymerase temporarily uses the newly synthesised strand of the sister chromatid as a template. When it has reached a point beyond the lesion site on the original template, it switches back to resume normal replication. In the process of daughter-strand gap repair, the DNA polymerase dissociates from the template in the vicinity of the lesion, after which the gap is repaired via a homologous recombination mechanism. After the damaged DNA has thus been bypassed, normal replication resumes beyond the damage. Both these processes are still poorly characterised and little is known about the proteins involved; apart from the Rad5 protein, only PCNA and DNA polymerase delta are reported to be involved (Torres-Ramos et al., 2002).

It has recently become clear that in the decision which subpathway of PRR to use, covalent modifications of PCNA, i.e. ubiquitination and SUMOylation play an important role. Specifically, mono-ubiquitination on lysine 164 of PCNA, a modification that depends on the Rad6-Rad18 complex (Hoege et al., 2002), results in the switch to the error-prone TLS pathway via the recruitment of pol η (Kannouche et al., 2004; Watanabe et al., 2004). In a Rad5- and Mms2-Ubc13-dependent reaction, a monoubiquitin moiety can subsequently be extended to a polyubiquitin chain, resulting in the selection of error-free PRR pathways (Hoege et al., 2002).

Mismatch repair

The replicative DNA polymerases (α, δ and ε) have relatively high fidelity, resulting in only 1 error per 10^5-10^6 nucleotides copied (Roberts and Kunkel, 1996). Still, thousands of mismatched base pairs will thus be introduced by copying the $6x10^9$ nt genome. Moreover, several TLS polymerases, which are much less accurate, may participate in replication past unrepaired lesions. If the mismatches are not removed, a next round of replication could fix them as mutations, resulting in possible impaired cellular functioning, cancerous cell formation or even cell death.

Mismatch repair (MMR) specifically deals with nucleotides misincorporated during replication, replacing the incorrect nucleotide in the newly synthesised strand with the correct one. MMR also removes insertion/deletion loops (IDL) resulting from polymerase slippage. Two heterodimers are responsible for recognition of the various MMR substrates: mismatched base pairs are recognised by the hMSH2-hMSH6 heterodimer, larger IDL are recognised by hMSH2-hMSH3, while both factors are capable of recognising single base insertions/deletions. After binding to their substrates, the hMSH2-containing heterodimers attract a heterodimer consisting of hMLH1 and PMS2; hMSH2-hMSH3 alternatively can attract an hMLH1-hMLH3 dimer. The hMLH1-containing heterodimers in turn couple the recognition of the mismatch or IDL with later steps, i.e.: 1) strand discrimination, still a poorly understood process in most organisms except *E. coli*; 2) excision, a step which (at least in *E. coli*) can remove hundreds of nucleotides up to the strand discrimination signal and which in mammals employs EXO1, PCNA and the proofreading exonucleases of DNA pol δ or pol ε; and 3) resynthesis by DNA pol δ or pol ε and associated factors. For a recent review on mismatch repair, the reader is referred to Kunkel and Erie (2005).