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

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CELLULAR RESPONSES TO DNA DAMAGE

DNA DAMAGE

Translation of genetic information into functional proteins as well as the inheritance of genetic code is essential for species survival. Alterations to the DNA induced by endogenous as well as exogenous agents threaten cellular survival by interfering with important processes such as replication and transcription. Spontaneous mutations are those intrinsic to the chemical nature of DNA in an aqueous solution and include deamination and the creation of abasic sites (Lindahl, 1993). Endogenous metabolism of the cell results in accumulation of reactive oxygen and nitrogen species as well as other metabolites which are able to damage DNA (De Bont and van Larebeke, 2004). Mutations are also the consequence of DNA damage induced by either mutagens present in the environment, such as UV radiation, dietary contaminants, air pollutants such as exhaust fumes and numerous chemicals. Exposure to X-rays and chemotherapeutic agents also cause DNA damage (Hoeijmakers, 2001). All organisms are exposed to damaging agents as part of everyday life. It is estimated that endogenous sources induce approximately 50,000 DNA lesions per cell per day in humans (Friedberg, 1995) whereas one hour of sunbathing is estimated to generate around 80,000 DNA lesions per cell (Mullaart et al., 1990). Metabolic cellular processes generate reaction products including active reactive oxygen species (ROS) and nitrogen species which cause oxidative modifications to DNA including base modifications (8-oxo-G, thymine gycols and cyclopurines) as well as single- and double-strand breaks (Cadet et al., 2003). In addition, ultraviolet light (UV) can induce the formation of helix-distorting lesions such as 6-4 photoproducts (6-4PP) and cyclopyrimidine dimers (CPD) (Wood, 1999). Ionizing radiation (IR) generates hydroxyl radicals introducing base damages which can lead to single- or double-strand DNA breaks (Cadet et al., 2003; Hoeijmakers, 2001).

CELLULAR RESPONSES TO DNA DAMAGE

Fortunately, eukaryotic and prokaryotic organisms possess a variety of genome maintenance mechanisms to protect themselves from constant assault by damage-inducing agents. Cells respond to DNA damage by a number of carefully coordinated processes such as, cell cycle checkpoint activation, induction of transcriptional programs, enhancement of DNA repair pathways, and chromatin remodeling and modification mechanisms (Hoeijmakers, 2007). When the level of damage is too severe, apoptosis is initiated. Nevertheless, none of these systems is perfect, and any remaining damage, or any damage repaired incorrectly, may play an important role in the induction of birth defects, the development of diseases such as cancer as well as aging.

DNA DAMAGE CELL CYCLE CHECKPOINTS

The cell cycle is organized into a series of sequential pathways; G1 followed by DNA replication or synthesis phase-S, followed by G2 and finally cell division or mitosis-M. DNA damage checkpoint systems are control mechanisms that delay cell-cycle progression in

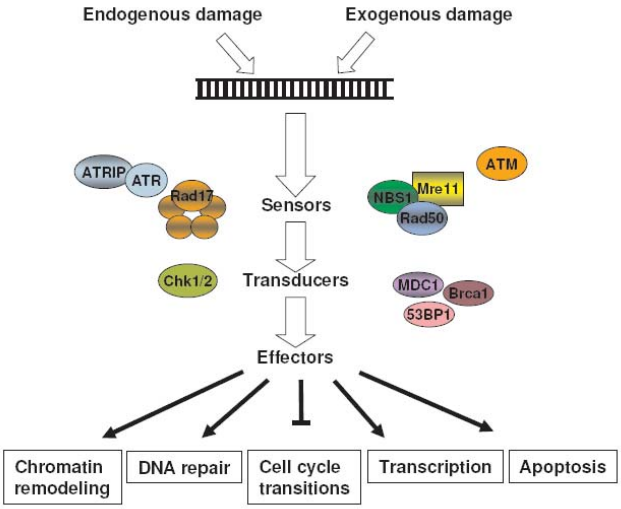

Fig. 1. Simplified representation of the DNA damage response. DNA damage is first detected by sensor proteins which in turn activate transducers in the signal cascade. These transducers then mediate the activation or inhibition of downstream effectors which can arrest the cell cycle, initiate DNA repair or induce apoptosis.

response to DNA damage or replication stress. These regulatory pathways govern the order and timing of cell cycle transitions ensuring completion of one cellular event prior to commencement of another which is essential for maintaining genomic integrity (Abraham, 2001; Bartek and Lukas, 2007). The delay in cell cycle progression enables cells to repair their DNA damage before the damage is passed on to daughter cells thereby ensuring faithful transmission of genetic information (Reinhardt and Yaffe, 2009; Bartek and Lukas, 2007). Mutations that inactivate DNA damage checkpoint genes result in disruption of the DNA damage signaling pathways and differences in the response of cells to DNA damage. DNA checkpoint control can occur in G1, S phase, or at the G2-M transition (Berkovich et al., 2007). The DNA damage response involves first sensing a signal and then transducing it to downstream effectors that regulate the activity of several downstream targets, including factors that regulate cell-cycle progression and DNA repair processes (Sancar et al., 2004). Sensors recognize the lesions themselves or remodelled chromatin structure that follows DNA damage (Downs et al., 2007). Transducers initiate a signal transduction cascade that propogate and amplify the signal and include ATM and ATR as well as p38 MAPK. Effector kinases such as ChK1 and ChK2 execute the specific cellular response (Bartek and Lukas, 2003).

The central components of the signal transduction response are the phosphoinositol-3-kinase-like protein kinases ATM (Ataxia telangiectasia), ATR (Ataxia telangiectasia and Rad3-related) and DNA-PK (Abraham, 2001). These transducers coordinate the initiation, amplification, and activation of the checkpoint through phosphorylation of many different targets. ATM is activated by DNA double strand breaks (DSBs) whilst ATR is activated by single stranded regions of DNA (ssDNA) and RPA exposed for example during UV damage repair and DNA replication fork stalling (Abraham, 2001; Zou and Elledge, 2003). Both kinases phosphorylate many of the same substrates (e.g. p53, Brca1, FANC-D2) and have distinct but overlapping signal transduction pathway functions. An important substrate

of ATM, ATR and DNA-PK in the vicinity of the damaged site is the histone H2AX, which represents a sub-family of histone H2A. Histone H2AX is an adaptor molecule of the DNA repair machinery which is phosphorylated at its C-terminus (designated γ-H2AX) thereby being a marker of DNA lesions (Fernandez-Capetillo et al., 2004). Additionally, γH2AX mediates DNA repair by promoting the maintenance of repair factors close to the DNA lesion (Bonner et al., 2008). ATM is recruited to DSBs via a sensor complex composed of Mre11-Rad50-Nbs1 (MRN complex). ATM phosphorylates histone H2AX, flanking the sites of DNA damage. MDC1, 53BP1 and BRCA1 accumulate at phosphorylated H2AX resulting in Chk2 activation (Canman, 2003). Chk2 which plays a role in controlling the activity of the Cdc25 phosphatases, a family of proteins intimately involved in the activation of various Cdk-Cyclin complexes (Bartek and Lukas, 2003). ATR exists as a heterodimer with ATRIP (ATR interacting protein) and is recruited to ssDNA via the Replication Protein A heterotrimer (RPA). The ATR/ATRIP complex is only activated and functional when the sensor Rad17 and 9-1-1 (Rad9, Rad1, and Hus1) complexes are loaded onto the DNA (Zou et al., 2002). Chk1 and Chk2 are checkpoint transducer serine/threonine kinases that function downstream in the DNA-damage checkpoint signaling pathway (Bartek and Lukas, 2007). Although Chk1 and Chk2 have overlapping roles, Chk1 is expressed during S and G2 where its activity is amplified in the presence of different types of DNA damage (Zhao et al., 2002). Chk2 is expressed throughout the cell-cycle and is also activated in the presence of DNA damage (Lukas et al., 2001).

In response to DNA damage, the G1 checkpoint prevents the cell from attempting to replicate damaged DNA by blocking cells from entering the S phase by inhibiting the initiation of replication (Massague, 2004). If damaged DNA or replication errors are sensed during S-phase, the S-phase checkpoint is activated. DNA synthesis is then slowed down in order to provide time for repair (Bartek et al., 2004). DNA damage incurred in G2 phase, or cells that have escaped G1 and S-phase checkpoints despite earlier DNA damage results in activation of the G2/M checkpoint to prevent entry into mitosis with damaged chromosomes (Taylor and Stark, 2001). There is currently no evidence that UV-induced lesions activiate ATM or DNA-PK. However, during S-phase, NER lesions generate stalled replication forks leading to accumulation of NER-related proteins and the generation of single-stranded streches of DNA. RPA, ATRIP and ATR accumulate triggering replication-stress signalling (Abraham, 2001; Ward et al., 2001; Jeggo and Lobrich, 2001). In addition to replication, nucleotide excision repair (NER) has also been implicated in ATR checkpoint activation (reviewed in Rouse and Jackson, 2002). These studies were conducted in yeast arrested in G1, providing evidence that NER is needed to generate a checkpoint-inducing signal in the absence of replication. It has been speculated that this signal is an RPA-coated, ssDNA-gapped structure (O'Driscoll et al., 2003; Giannattasio et al., 2004). Whether or not NER directly contributes to activation of the ATR-dependent checkpoint in higher eukaryotes is unclear. However, Both ATR and its associated protein ATRIP have been shown to translocate to punctate nuclear foci following treatment of cells with UV (Ward et al., 2004), suggesting that there is an ATR-mediated DNA damage response in normal mammalian cells. The MAPKAP kinase-2 (MK2) is a transducer kinase which is downstream of the stress-response p38 MAPK pathway. MK2 is directly involved in

phosphorylating effectors CDC25B and C, and in maintaining G1, S, and G2 checkpoints triggered by UV-induced DNA damage, cisplatin, camptothecin and doxorubicin (Manke et al., 2005).

Together the transducers ATM/Chk2, ATR/Chk1 and MK2 phosphorylate a variety of effector proteins, such as p53 and the CDC25 phosphatases which transmit the DNA damage signal downstream, eventually arresting the cell cycle, initiating DNA repair, or, if necessary, apoptosis. More detailed information on the DNA damage checkpoint signaling pathways at the molecular level can be found in recent reviews by Reinhardt and Yaffe (2009) and Lazzaro et al., (2009).

DNA REPAIR

Several different DNA repair pathways have been identified which each focus on a specific category of DNA lesions. These repair systems also differ in the way that the damage is recognized which is dependent on the severity of DNA damage (Hoeijmakers, 2001). To date, more than 130 DNA repair enzymes have been identified which ensure genomic integrity (Hoeijmakers, 2007; Schumacher et al., 2008). Once damage is repaired and chromatin is restored, cells are able to proceed through the cell cycle. The main repair pathways in mammals are nucleotide excision repair (NER), Base excision repair (BER), Homologous recombination (HR), Non-homologous End-joining (NHEJ) and Mismatch repair (MMR) (Friedberg, 2003; Hoeijmakers, 2001).

Nucleotide Excision Repair

Nucleotide excision repair (NER) is one of the most versatile DNA repair systems in humans. NER eliminates bulky DNA damage that leads to distortions of the DNA helix. In fact, this repair pathway is essential for the repair of UV-induced lesions, such as CPDs and 6-4PPs. NER is a multi-step process which requires around 30 different proteins and removes DNA damage by dual incision of the damaged strand. NER is divided into two sub-pathways which allow either efficient removal of lesions which block ongoing transcription, known as transcription-coupled repair (TCR), or removal of lesions at any other position in the genome which is known as global genome repair (GGR) (Tornaletti and Hanawalt, 1999). Since my work has focused on a better understanding of the NER pathway, the subsequent chapters in this thesis describe NER in greater detail.

Base Excision Repair and Single-strand break repair

Base excision repair (BER) is a major pathway for the repair of a wide variety of DNA base damages which include oxidative DNA damage (8-oxoguanine, thymine glycols) alkylation products, and single strand breaks (Beckman and Ames, 1997; Fortini et al., 2003). BER is classified into 2 sub-pathways: short-patch and long patch repair. Short-patch BER is a mechanism whereby only 1 nucleotide is replaced, whereas in long patch BER, 2-13 nucleotides are replaced. Various DNA glycosylases have been identified, each with specific affinity for a subset of lesions. These gycosylases first recognize specific damaged bases and excise them from the genome, initiating both long-patch and short-patch BER.

Removing the damaged base, followed be nuclease mediated strand incision results in an abasic site (AP site) (reviewed in Dodson and Lloyd, 2002) which is subsequently refilled by means of DNA synthesis by DNA polymerase pol β. Finally either the XRCC1-DNA Ligase III complex or DNA Ligase I allows efficient ligation restoring the integrity of the helix (Wilson, III and Bohr, 2007; Almeida and Sobol, 2007).

Double-strand break repair

Double-stand breaks (DSBs) are among the most lethal of all DNA lesions since their inefficient or inaccurate repair results in genetic rearrangements that can lead to cancer or cell death. Efficient repair of DSBs is therefore essential for genome stability (Rich et al., 2000). DSBs are caused by a variety of sources including ionizing radiation, certain genotoxic chemicals, endogenously generated ROS and replication of single-stranded DNA breaks (Hoeijmakers, 2001). V(D)J recombination also generates DSBs during rearrangement of genes encoding B cell immunoglobulins and T cell receptors. DSBs differ from most other types of DNA lesions in that they affect both strands of the DNA duplex and therefore prevent use of the complementary strand as a template for repair as seen for example in NER. DSBs are repaired by 2 major mechanisms: homologous recombination (HR) and non-homologous end-joining (NHEJ). The choice of which pathway to utilize appears to be largely influenced by stage within the cell cycle at the time of damage acquisition (reviewed in Delacote and Lopez, 2008; Shrivastav et al., 2008). The NHEJ pathway simply repairs DSBs by re-ligating their 2 ends together, but bases may be added or lost as it occurs making it an inaccurate process. The core NHEJ machinery is known to be composed of 3 core complexes: MRN, Ku and the DNA ligase complex although the order of action of these complexes has not yet been fully established. Nevertheless, it is thought that MRN and Ku complexes bind DSBs shortly after DSB formation, inhibiting their degradation. In addition to bridging the DSB ends together, Ku and MRN also play crucial roles in recruiting, stabilizing and stimulating the ligase complex (Scott and Pandita, 2006; Weterings and Chen, 2008). After replication, HR acts by initiating a series of complex DNA transactions between the identical sister chromatid to properly align the broken ends inserting missing information. If the DNA template used for repair is not identical to the original DNA sequence present at the DSB, HR can cause mutations or more severe genome rearrangements (Szostak et al., 1983). (Reviewed in Natarajan and Palitti, 2008).

Mismatch repair

The mismatch repair (MMR) pathway is a multi-step process which plays an essential role in the correction of mismatches such as those due to replication errors. Such base-base mismatches and insertion/deletion loops result from DNA polymerase mis-incorporation of nucleotides and template slippage (Hsieh and Yamane, 2008). Failure of mismatch correction by cellular repair systems or misdirected repair to the strand containing the original and correct DNA sequence will ultimately lead to genetic mutations (Modrich, 2006). MMR recognizes and corrects all base-base mismatches and small insertions/

deletions and therefore increases the fidelity of DNA replication by up to 1000 fold. Cells harboring mutations in MMR proteins suffer from an increased frequency of spontaneous mutations emphasising the importance of this repair pathway (Kunkel and Erie, 2005). MMR is described in the review by Hsieh and Yamane (2008).

TRANSLESION SYNTHESIS

If DNA damage still persists despite the above mentioned repair processes replication blockage can occur. One way to circumvent this block is by "damage avoidance", using recombinational mechanisms to copy genetic information from the undamaged sister duplex. The alternative is to incorporate nucleotides opposite the damage, a process-designated as translesion synthesis (TLS) (Jansen et al., 2007). TLS makes use of special DNA polymerases that are able to bypass specific types of DNA damage in order to avoid replication blockage.TLS polymerases η, ι, κ and Rev1 (Y-family polymerases), as well as polymerase ζ (B-family) have been identified, each with their own specificities. These polymerases take over synthesis in order to bypass the injured DNA lesion. TLS is reviewed by Green and Lehmann (2005) and Jansen et al., (2007).