Tracking DNA double-strand breaks

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INTRODUCTION
Cells continuously suffer from DNA damage. Each cell in an organism has to cope with 10,000-100,000 DNA lesions per day. These include single-strand breaks (SSBs), double-strand breaks (DSBs) and oxidative lesions. DNA damage is sensed and repaired by a very efficient and evolutionary highly-conserved set of pathways called the DNA-damage response (DDR). DSBs are among the most hazardous type of DNA lesions. A single unrepaired DSB is sufficient to induce cell death or, alternatively, may drive a cell into an irreversible state of dormancy known as senescence. Wrongly-repaired DSBs may have serious consequences for the organism as a whole, as they can lead to genetic instability and can induce cancer.

Thus, DSBs play an important role in carcinogenesis but, at the same time they can be used as a means for killing cancer cells. There are many ways to induce DSBs and different agents induce different types of DNA damage. Therefore, the choice of agent used to induce damage, determines to a great extent the cellular response. This implies that induction of DSBs is a critical step in all investigations in which the nature or the consequences of DSBs are studied. In the present thesis, two methods are presented to induce DSBs in specific areas of the cell nucleus. The first one is based on the irradiation of cells with α-particles (Chapter 2). When using this technique, small numbers of DSBs are induced. This can be a disadvantage for cell biological analysis of DSB response proteins, particularly, when the protein of interest is present at DSBs in low copy numbers that cannot be visualized. To circumvent this, we developed a second method using ultra-soft X-rays to induce large numbers of DSBs in a small area of the cell nucleus by microbeam irradiation (Chapter 3).

Both methods facilitate visualization of DSBs using fluorescence microscopy and the methods are suitable to study live cells as well as fixed cells. In Chapter 4 and 5, supporting techniques are described, that facilitate application of the DSB induction methods.

The α-particle method proved to be a useful tool for the analysis of the dynamic behaviour of DSB-containing chromatin domains (Chapter 6). Furthermore, the method was applied to determine whether or not the human telomeric protein TRF2 is recruited to DSBs (Chapter 7). The ultra-soft X-ray method was used to study the accumulation of HP1 at the sites of DSBs (this study was published in a recent paper, but is not included in the present thesis).

**SOURCES OF DSBs**

A DSB is a break in double-stranded DNA in which both strands in the double helix are cleaved. DSBs are induced by exogenous agents, such as chemicals and X-rays, but can also result from endogenously-generated reactive oxygen...
species (ROS) and mechanical stress on the chromosomes. In addition, DSBs can be induced during the replication process, when a replicating fork meets a SSB or during repair of interstrand crosslinks. DSBs can also occur during programmed rearrangements, such as developmentally-regulated rearrangements and immune class-switch recombination, a process that changes production of antibodies from one class to another in B cells.

**DNA DAMAGE RESPONSE**

DNA damage often causes a transient arrest of cell-cycle progression at specific checkpoints. The arrest provides the cell time for repair. When the damage is properly fixed, cells resume proliferation, but when the damage is too severe, cells undergo programmed cell death (apoptosis) or die in mitosis. Alternatively, cells go into an irreversible state of cell-cycle arrest, called cellular senescence. Wrongly-repaired DNA may lead to mutations or chromosome aberrations. A mutation is a change in DNA sequence. Chromosomal aberrations such as deletions and chromosome exchanges can be the results of DSBs. Both mutations and chromosome aberrations are associated with carcinogenesis, for instance through inactivation of tumour-suppressor genes or activation of proto-oncogenes.

The DDR has two functions that are linked: one, prevention of propagation of damaged DNA or corrupted genetic information into daughter cells and, two, repair of damaged DNA to maintain genome integrity. There is no single repair process capable of handling all types of DNA damage. The major, narrowly-interwoven, DNA repair systems active in mammalian cells are: base-excision repair (BER), nucleotide-excision repair (NER), mismatch repair (MMR), homologous recombination (HR) and non-homologous end joining (NHEJ). BER targets chemically-altered bases in DNA that often arise from endogenous agents. Therefore, BER plays an important role in the prevention of mutagenesis. It is also involved in repair of SSBs. NER is active in repair of helix-distorting lesions, which are often caused by exogenous agents. NER removes bulky adducts, such as UV-induced photoproducts and cyclobutane pyrimidine dimers. There are two distinct NER pathways: the transcription-coupled repair pathway, which removes lesions from actively-transcribed DNA and the global repair pathway, which removes lesions throughout the genome. Both BER and NER only repair lesions in one of the DNA strands by removal of damaged parts and fixing the resulting single-stranded gap using the intact strand as a template. MMR detects and replaces wrongly paired, mismatched bases in newly replicated DNA. If left uncorrected, mismatches may lead to stable mutations. The repair mechanisms for DSBs are discussed below.
The observation that DNA lesions are repaired through damage-specific pathways, underlines the importance of characterizing the damage spectrum induced by a damage inducing agent.

SIGNALLING IN RESPONSE TO DSBs

DSBs are difficult to repair, because both DNA strands are affected. One of the early sensors of a DSB is the MRE11-Rad50-NBS1 (MRN) complex. It recruits the apical kinase ataxia telangiectasia mutated (ATM), one of the key factors in DDR. ATM is autophosphorylated and causes the phosphorylation of histone H2AX. The phosphorylated form of H2AX (γH2AX) recruits additional ATM and local ATM activity induces the spreading of H2AX phosphorylation. This feedback mechanism is mediated by two DDR mediators (signal boosters) that are attracted to γH2AX: damage checkpoint 1 (MDC1) and p53-binding protein 1 (53BP1). γH2AX attracts other DDR factors.

Levels of ATM at the site of a DSB, above a certain threshold, engage kinases CHK1 and CHK2. These molecules diffuse freely in the nucleus and are responsible for DDR signalling in nuclear regions away from the DNA damage site. P53 and CDC25 are activated by CHK1 and CHK2. CDC25 inactivation causes a rapid cell-cycle arrest, whereas p53 induces the transcription of p21, a cyclin-dependent kinase inhibitor, which results in a stable cell-cycle arrest allowing repair of DNA damage. Cell-cycle arrest is in principle transient, but when the damage is too severe, cells either enter senescence or undergo apoptosis. It is still unclear what determines the selection between apoptosis and senescence. It may be determined by cell type and the intensity and/or nature of the damage.

DSB REPAIR

The two main pathways for DSB repair are HR and NHEJ. They operate with different speed and accuracy. NHEJ is active throughout the cell cycle and HR is the preferred pathway of DSB repair in S and G2 phase, when a second identical DNA sequence is available at the sister chromatid after replication.

HR is error-free, because the identical sequence is used as a template to repair DNA. Repair may take several hours (up to 6 hours) to be completed. The main proteins involved in this pathway are: RPA, BRCA2, Rad51, Rad52, Rad54 and XRCC2. The ends of the break are resected in a process involving the MRN complex to generate 3’-tailed DNA. Rad51 then, facilitated by RPA, forms nucleoprotein filaments on the exposed ends. This process is initiated
by a Rad51-BRCA2 complex. The next step is DNA strand invasion with a homologous sequence. This is mediated by Rad51 and Rad54. The invading DNA is extended to generate intermediates with crossed DNA strands. Repair is completed by resolving these junctions and ligation.

NHEJ directly joins open DNA ends, does not require sequence homology and is, therefore, active in all cell-cycle phases. Obviously, it plays a major role in the repair of DSBs in pre-replicated DNA, because there is no second identical DNA sequence available to serve as a template. It is considered to be more error-prone than HR; sometimes loss or gain of nucleotides occur, and it is held responsible for producing chromosome translocations more frequently than HR. However, NHEJ allows cells to quickly resume proliferation, since the majority of DSBs is repaired within 30 min. The “quick and dirty” aspect of NHEJ is an important strategy of a cell to survive. Already one unrepaired DSB can be lethal, due to loss of a piece of chromosome in the next mitosis. Although NHEJ is error-prone, the chance that mis-repair occurs in DNA that contains coding genes or regulatory regions is limited, as only a few percent of DNA contains these regions.

Broken DNA ends are recognized by a complex containing Ku70 and Ku80 that recruits the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs). Ligation is mediated by XRCC4/ligase IV heterodimer and associated proteins. Besides the DNA-PK NHEJ pathway an alternative backup NHEJ pathway (B-NHEJ) has recently been described that is independent of DNA-PK. It has been suggested that the B-NHEJ pathway is slower than the DNA-PK-dependent pathway.

DNA REPAIR AND CHROMATIN STRUCTURE

Organization of DNA in the cell nucleus is important for the repair of DSBs, since it plays a role in damage recognition and determines access of repair-related proteins to DNA.

In eukaryotic cells, DNA is organized in a nucleosomal structure, where approximately 146 base pairs are wrapped around histone octamers. Each octamer consists of two molecules of histone H2A, H2B, H3 and H4. Additional variants of the histones may be present in the nucleosomes. DNA wrapped around octamers form “beads on a string” filaments, which are coiled into 30 nm fibre structures. These fibres are folded into a higher order configuration that is poorly understood. DNA plus associated proteins is called chromatin.

The nucleosome is a dynamic structure that allows a variety of proteins access to the DNA. Chromatin-DNA interactions are regulated by modifications of the N- and C-ends of the histone tails. These modifications function
as a ‘histone-code’, that extends the information of the genetic code. Histone modifications including phosphorylation, methylation, acetylation and ubiquitylation play an important role in DNA repair. They can change chromatin compaction to allow repair-related proteins access to damaged DNA and they can attract proteins that play a role in repair. An example of a repair-related histone modification is phosphorylation of histone H2AX (see above). Another example is methylation of histone H4, a modification necessary to attract 53BP1. Because 53BP1 also binds to histone deacetylase 4 (HDAC4), it has been suggested to play a role in coordinating DSB repair with chromatin structure and function.

VISUALIZATION OF DSBs

DDR proteins accumulate at sites of DNA damage in microscopically easily recognizable spots called irradiation-induced foci (IRIF). IRIFs are dynamic structures containing thousands of copies of repair-related proteins. It is not fully understood how the DDR pathway benefits from the massive amounts of proteins at DSB sites, but the general consensus is that it is catalytically profitable. IRIFs can be relatively large, e.g. γH2AX-containing chromatin domains span several megabases on both sides of a DSB. They can also be small, e.g. when they represent repair-related proteins operating in close vicinity to DSBs such as Rad51.

IRIFs also participate in chromatin restructuring, in the vicinity of DNA damage, to increase the accessibility of DNA lesions for repair factors. Protein assembly in DSB-flanking chromatin is important since it protects the integrity of epigenetic information encrypted in these regions.

IRIFs can be visualized either by immunostaining or by fluorescently-labeled DDR proteins in living cells. Although these foci are often accepted as proof of DSBs, it should be noted that they are a read-out of DDR proteins and not of DNA damage. DNA damage may be present without visible foci, because of impairment of upstream DDR signalling. The opposite is also possible, DDR proteins may accumulate in the absence of DNA damage in structures resembling IRIFs.

TELOMERES

The 2009 Nobel prize in Physiology or Medicine was awarded to Elizabeth Blackburn, Carol Greider and Jack Szostak who have solved a major problem in biology: how the chromosomes can be copied from end to end during cell division and how they are protected against degradation. The answer is
to be found in the ends of the chromosomes, the telomeres. Telomeres are nucleoprotein structures located at the ends of chromosomes of most eukaryotic organisms and some prokaryotes. Their major function is to disguise natural chromosome ends so that they are not recognized as DSBs and thus they protect chromosome ends from unwanted actions of repair-related proteins. They are often compared to the tips at the end of shoelaces, protecting the laces from unraveling.

During each cell division telomeres lose base pairs. The explanation for this phenomenon is the “end-replication problem”: the DNA replication machinery cannot replicate DNA up to the end of the chromosomes. Telomere length is an indicator of the number of cycles a cell has gone through. In fact, telomeres set a limit to the number of cycles that cells can run. When a telomere is critically short, a cell usually goes into senescence. When cells divide after having lost their telomeres, the result is chromosome ends sticking to other chromosome ends with genomic instability as a consequence.

Male germ line cells, activated lymphocytes and some stem cell populations have active telomerase, a ribonucleoprotein, composed of an RNA template (hTERC) and a reverse transcriptase catalytic subunit (hTERT). Telomerase is capable of synthesizing and elongating telomeres and is active until birth or shortly thereafter. The enzyme is reactivated in 90% of human cancers. Some cancer cells and immortalized cell lines maintain telomere length by employing a recombination-based alternative pathway: alternative lengthening of telomeres (ALT).

Telomeres are composed of repetitive DNA ending in a G-rich 3’ single-stranded overhang, that loops back and integrates in the telomeric duplex DNA. This loop is essential for telomere maintenance and capping. A complex of six core proteins named shelterin specifically associates with telomeric DNA. The function of the Shelterin complex is to maintain telomere length and end-capping. The complexes include telomeric repeat proteins: TRF1, which regulates telomere length assisting telomerase, and TRF2, which models telomeres in a loop structure. TRF2 deficiency results in massive telomere dysfunction characterized by an ATM-mediated damage response, chromosome fusions maintaining telomere sequences (telomere fusions), accumulation of DNA repair factors at telomeres, and apoptosis. Besides shelterin proteins, other proteins are located at telomeres as well. Compared to shelterin proteins, they are less abundant and often only transiently associated. Most of these proteins also have non-telomeric functions related to DNA damage signaling and repair and are recruited by TRF1 and TRF2. Examples of these accessory proteins are: KU proteins, MRN complex, ATM and DNA-PKcs. The exact function of these proteins at telomeres is not clear, but most of them seem to
be involved in telomere maintenance. This is supported by the observation that telomeres in DNA-PKcs-deficient cells can fuse, but can also fuse to DSBs to produce chromosome translocations \(^{51,55}\).

The presence of DSB repair proteins at telomeres has brought up the converse question, whether telomere proteins may have functions in DSB repair. In the study described in Chapter 7, we introduced DSBs by irradiation with \(\alpha\)-particles to determine whether or not the human telomeric protein TRF2 is recruited to DSBs.

**DSB INDUCTION IN EXPERIMENTAL SETTINGS**

Ionizing radiation (IR) is the most commonly used tool to induce DSBs \(^{56}\). The characteristics of IR make it a powerful agent to induce DSBs: the damage spectrum is well-characterized and the number of DSBs per cell can be tightly controlled. IR is an ideal tool for experiments in which large populations of irradiated cells are needed, for example to study cell survival using the clonogenic assay \(^{57}\), because cell cultures can be irradiated homogeneously.

Cesium 137 (\(^{137}\)Cs) is often used to irradiate cells. \(^{137}\)Cs emits \(\gamma\)-rays, electromagnetic radiation, which interacts with orbital electrons. As a consequence, electrons can be ejected from atoms (ionization) or can be raised to higher energy levels within an atom or molecule (excitation). Ejected electrons can cause further ionization and/or excitation. Ionization and excitation lead to disruption of chemical bonds and formation of free radicals in water molecules. The density of ionization is described as linear energy transfer (LET).

\(\gamma\)-Rays have a mean LET of 0.3 keV \(\mu\)m\(^{-1}\) and are considered to be low LET radiation. \(\alpha\)-Particles have a LET of approx. 100-200 keV \(\mu\)m\(^{-1}\) and are an example of high LET radiation. \(\alpha\)-Particles produce more localized ionization leading to more severe DNA damage.

Although irradiation of cells with IR causes not only damage to DNA, but also to other molecules, it is well-accepted that DNA is the critical target for radiation-induced cell death \(^{58,59}\). Damage to other molecules has little consequence, because there are multiple copies of most molecules and most undergo a rapid turnover. In contrast, DNA is only present in two copies and has limited turnover. Furthermore, it is the largest molecule and thus the largest target.

DNA is damaged by IR through direct effects on the molecule itself or indirectly by radicals. IR induces base damage, SSBs and DSBs. In mammalian cells, at an absorbed dose of 1-2 Gy (\(\gamma\)-rays) approx. 40 DSBs, 1000 SSBs and more than 1000 damaged bases are induced. SSBs and base damage outnumber DSBs, but they are repaired very efficiently and do not contribute
to cell death \(^{60,61}\). Even when high numbers of SSBs are induced by hydrogen peroxide, cell death is rare. Therefore, it is generally accepted that DSBs are the critical lesions for radiation-induced cell death \(^{62}\).

An alternative way to induce DSBs is by using radiomimetic chemicals. For example, bleomycin generates DSBs by producing free radicals and etoposide induces DSBs at specific locations by inhibition of topoisomerase II \(^{63-65}\). The damaging effect of these compounds depends on diffusion processes. In contrast, IR penetrates tissues and cells easily and ionization events occur very fast, within microseconds.

**LOCAL INDUCTION OF DSBs**

Fluorescent protein technology has opened the field of DSB repair studies to live cell microscopy. DNA repair-related proteins genetically tagged with green fluorescent protein or its variants allow the study of cellular responses to DSBs \(^{37,66-68}\). Local induction of DSBs is the method of choice in live cell experiments, because it allows the determination of repair-related proteins at sites of DSBs, their local concentrations, the speed of recruitment and their residence time. Cellular responses to DSBs are fast. Within seconds, the DDR pathway is activated \(^{7,69,70}\), implying that DSB induction preferably has to take place under the microscope to minimize the time between DSB induction and subsequent monitoring of the cellular response to DSBs.

The commonly-used IR techniques with X-rays or \(\gamma\)-rays are not very suitable for live cell experiments. It is not possible to visualize recruitment of repair proteins in real time, because irradiation cannot take place under the microscope. Furthermore, DSBs are induced randomly throughout the genome and not in a localized manner. DSBs induced at random locations throughout the nucleus are hardly distinguishable from “background” DSBs, such as DSBs originating from stalled replication forks.

In recent years, several methods have been developed to locally induce DSBs and to analyze the subsequent repair processes in living cells \(^{71}\). Lasers used in confocal or microdissection microscopes have been adapted to induce DNA damage in a pre-defined localization in a cell nucleus. These techniques are based on the pre-sensitization of DNA with halogenated thymidine analogs or the DNA dye Hoechst 33258 \(^{72}\). However, laser microbeam irradiation presents some major drawbacks: the damage spectrum is not very well-characterized and results in the response of various repair pathways. Furthermore, proteins involved in the repair of a variety of lesions, such as xeroderma pigmentosum (XPC), show a kinetic behaviour that depends on the type of damage that is induced \(^{73}\). Finally, the dose that is needed to recognize the damaged area is
relatively high. Two-photon lasers \(^{74}\), in particular, deposit an extreme amount of energy in a confined nuclear volume, resulting in large amounts of photothermal damage \(^{75}\). As is described in Chapter 7, analysis of recruitment of proteins at DSBs using dual photon irradiation may lead to debatable results.

Laser microbeam irradiation without pre-sensitization \(^{76,77}\) induces more specific DNA damage, but requires even higher laser energy and can induce undefined damage to other cellular structures as well.

The introduction of rare restriction sites into the genome followed by controlled expression of the relevant endonuclease is another way to induce DSBs in a pre-defined localisation. This method was developed first in yeast \(^{78,79}\), but has also been adapted for its use in mammalian cells \(^{80}\). DSBs can be monitored in time by flanking the restriction sites with tet- or lac-operator sequences and expression of fluorescently tagged tet- or lac-binding fusion proteins. A drawback of this technique is the relatively long period of time (approx. 30 min) between induction of endonuclease expression and actual DSB formation.

**LINEAR TRACKS OF DSBs**

In Chapter 2, we present a method to induce DSBs in specific areas of the cell nucleus, that has the advantages of IR, but circumvents the drawbacks associated with the above-mentioned techniques. Cells are exposed to \(\alpha\)-particles from a small Americium source placed alongside the cells. Each \(\alpha\)-particle traversing the cell nucleus induces a linear easy-recognizable track of DSBs: approximately 20 DSBs per 10 \(\mu\)m track length, corresponding with a dose of only 0.5 Gy in a 500 \(\mu\)m\(^3\) nucleus. Most cells can repair the majority of 20 DSBs successfully.

DSBs are not induced in a pre-defined area, but this is compensated by the fact that the linear tracks of DSBs are easy to recognize under the microscope. DSBs can be visualized by immunodetection or labelling in the living cell by fluorescent DSB response markers. Recruitment of repair proteins can be visualized in real-time, because the irradiation can take place under the microscope.

Analysis of the spatial distribution of DSBs-containing chromosome domains at various time intervals allowed us to study the dynamic behaviour of DSBs (Chapter 6).

**SOFT X-RAYS**

The \(\alpha\)-particles technique described above was designed to irradiate cells with a low dose in a specific area. However, when a protein of interest accumulates
at DSBs in low concentrations, it may be difficult to detect IRIFs. These experiments would benefit from a method allowing local IR irradiation with a high dose. Therefore, we developed a technique suitable for this type of experiments.

In Chapter 3, we describe a soft X-ray microbeam system for local DSB induction. Soft X-rays are also referred to as extended UV (wavelength 20 nm) and are an example of low LET radiation. The damage spectrum of soft X-rays is well characterized. UV-specific lesions are not generated but base damage, SSBs and DSBs are induced. The system can be operated at a high dose rate: approx. 50 DSBs per second can be induced simultaneously in a large number of cells.

A simple, but effective way to induce local damage is realized by placing a micro-mesh filter between radiation source and cell culture. This design was already successfully applied in combination with UV light to locally induce UV lesions. In our soft X-ray system, metal mesh filters are used to produce microbeams. The use of filters with different pore sizes (2.5 – 5 µm), spaced at different distances, allows control of the size of the exposed area and the number of exposed areas in irradiated cells.

The method was successfully applied to study the role of heterochromatin protein 1 (HP1) in the DNA damage response. In this study, the soft X-ray method was applied in fixed cell experiments, but the combination of soft X-ray irradiation with an upright fluorescence microscope equipped with a water-dipping objective should allow imaging of live cells during irradiation.

**SUPPORTING TECHNIQUES**

Because soft X-rays as well as α-particles cannot penetrate water, glass coverslips or cell culture plastics, cells have to be cultured on an ultra-thin mylar membrane and irradiated from below. This approach presents a problem, since many anchorage-dependent cells do not readily attach and spread on mylar. Cells that are not properly attached are more sensitive to IR. Furthermore, cell loss is considerable due to cell death and clumping of cells. A technique that we originally developed to promote cell attachment on glass cover slips turned out to be very useful to promote attachment of cells to mylar. This technique, based on glow-discharged carbon coating of a cell culture substrate, is described in Chapter 4.

Both DSB induction methods described in the present thesis are designed to induce DSBs that can be visualized using fluorescence microscopy. In the studies described in Chapter 6 and 7, DSBs are visualized by immunodetection. However, both induction methods can also be used in combination with live
cell microscopy, a technique that is becoming increasingly important because it allows the study of proteins in their natural environment, the living cell. Live cell experiments are the logical continuation of the study described in Chapter 6. Therefore, we describe methods in Chapter 5 that can be used to visualize and analyze the spatio-temporal dynamics of DSB-containing chromatin domains in mammalian cell nuclei in living cells.

MOBILITY OF DSBs

In Chapter 6, we present indications that chromatin domains that contain DSBs are mobile and can fuse. This is an important observation, because the consequence of two or more DSBs coming together before they are repaired, can be the rejoicing of wrong DNA ends and the formation of chromosome exchanges. Chromosomes occupy distinct spaces in the cell nucleus. This finding combined with the observation that IR, and α-particles in particular, induce complex chromosome exchanges, involving multiple chromosomes, suggests that mobility of DSBs plays a role in the formation of chromosome exchanges. Chromosome exchanges can be lethal. For example, a dicentric chromosome containing two centromeres causes mechanical problems during mitosis leading to cell death. On the other hand, a reciprocal translocation (one chromosome fragment exchanged for another) that does not involve an essential region of the genome, can be passed on to daughter cells and may eventually drive cells to malignant transformation.

Without irradiation, mobility of DSBs may not play a significant role, since DSBs occur at low frequency and the distances between DSBs are mostly too large for the formation of chromosome exchanges. However, the dynamic behaviour of DSBs may play a significant role when IR or radiomimetic chemicals are used in cancer therapy to kill cancer cells, thereby inducing large amounts of DSBs in cells of the surrounding normal tissues. These DSBs may lead to chromosome exchanges contributing to the risk of new cancers in patients treated with radiotherapy.

TRF2 RECRUITMENT TO DSBs

It is now well-established that repair-related proteins can be found at telomeres, but the question remains whether telomere proteins are recruited to DSBs. More specifically, Bradshaw et al. observed that TRF2 is recruited to DSBs that were induced by laser microbeam irradiation using a dual photon laser. This led to the conclusion that TRF2 plays a role in DDR. In the study presented in Chapter 7, we demonstrate that this conclusion is debatable, since TRF2 accumulation could not be detected at DSBs induced by induction techniques with better characterized damage spectra.
In summary, the present thesis describes novel approaches to study DSBs microscopically in order to unravel cellular processes that occur after induction of DSBs.

**REFERENCE LIST**

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CHAPTER 1

INTRODUCTION


CHAPTER 1
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