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Risks from low dose/dose rate radiation: what an understanding of DNA damage response mechanisms can tell us

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Abstract: Abstract—The DNA damage response (DDR) mechanisms represent a vital line of defense against exogenous and endogenous DNA damage to enhance two distinct outcomes, survival and the maintenance of genomic stability. The latter is critical for cancer avoidance. DDR processes encompass repair pathways and signal transduction mechanisms that activate cell cycle checkpoint arrest and apoptosis. DNA double strand breaks (DSBs) represent important radiation-induced lesions. The major DSB repair pathways are DNA non-homologous end-joining (NHEJ) and homologous recombination (HR) and ataxia telangiectasia mutated (ATM) activates the DSB signalling response. To evaluate the ability of these pathways to protect against low doses or dose rate radiation exposure, it is important to consider the fidelity of DSB repair and the sensitivity of checkpoint arrest and apoptosis. Radiation-induced DSBs are more complex than endogenously-induced DSBs, with the potential for multiple lesions to arise in close proximity. NHEJ, the major DSB repair pathway, cannot accurately reconstitute sequence information lost at DSBs. Both pathways have the potential to cause translocations by rejoining erroneous DNA ends. Thus, complete accuracy of repair cannot be guaranteed and the formation of translocations, which have the potential to initiate carcinogenesis, can arise. Additionally, the G2/M checkpoint has a defined sensitivity, allowing some chromosome breakage to occur. Thus, genomic rearrangements can potentially arise even if the G1/S checkpoint is efficient. The sensitivity of apoptosis is currently unclear but will likely differ between tissues. In summary, it is unlikely that the DDR mechanisms can fully protect cells from genomic rearrangements following exposure to low doses or dose rate radiation.

**RISKS FROM LOW DOSE/DOSE RATE RADIATION:
WHAT AN UNDERSTANDING OF DNA DAMAGE RESPONSE
MECHANISMS CAN TELL US**

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Abstract—The DNA damage response (DDR) mechanisms represent a vital line of defense against exogenous and endogenous DNA damage to enhance two distinct outcomes, survival and the maintenance of genomic stability. The latter is critical for cancer avoidance. DDR processes encompass repair pathways and signal transduction mechanisms that activate cell cycle checkpoint arrest and apoptosis. DNA double strand breaks (DSBs) represent important radiation-induced lesions. The major DSB repair pathways are DNA non-homologous end-joining (NHEJ) and homologous recombination (HR) and ataxia telangiectasia mutated (ATM) activates the DSB signalling response. To evaluate the ability of these pathways to protect against low doses or dose rate radiation exposure, it is important to consider the fidelity of DSB repair and the sensitivity of checkpoint arrest and apoptosis. Radiation-induced DSBs are more complex than endogenously-induced DSBs, with the potential for multiple lesions to arise in close proximity. NHEJ, the major DSB repair pathway, cannot accurately reconstitute sequence information lost at DSBs. Both pathways have the potential to cause translocations by rejoining erroneous DNA ends. Thus, complete accuracy of repair cannot be guaranteed and the formation of translocations, which have the potential to initiate carcinogenesis, can arise. Additionally, the G2/M checkpoint has a defined sensitivity, allowing some chromosome breakage to occur. Thus, genomic rearrangements can potentially arise even if the G1/S checkpoint is efficient. The sensitivity of apoptosis is currently unclear but will likely differ between tissues. In summary, it is unlikely that the DDR mechanisms can fully protect cells from genomic rearrangements following exposure to low doses or dose rate radiation.

Keywords: radiation, low level; radiation damage; radioprotection; DNA

INTRODUCTION

DNA damage response (DDR) mechanisms play a vital role in enhancing survival to DNA damaging agents but, as importantly, they also serve to maintain genomic stability in the face of exogenous and endogenous DNA damage. The maintenance of genomic stability may not significantly influence survival, but it is of importance to the well-being of an organism and is critical for cancer avoidance. The DDR processes have most likely evolved to handle continuous exposure to low levels of DNA damage rather than an acute, high dose exposure scenario. Despite this, there exists only a superficial understanding of how the efficiency and accuracy of the processes depend on the level of DNA damage. An evaluation of the impact of low doses of ionizing radiation (IR) is further complicated by the fact that radiation damage has important distinctions to the damage that arises endogenously. Notwithstanding these limitations in our knowledge, the DDR mechanisms clearly play an important role in preventing the onset of carcinogenesis from environmental or endogenous mutagens since most human syndromes caused by defects in DDR mechanisms are characterised by pronounced predisposition to cancer. Indeed, even individuals carrying mutations in just one allele of certain DDR genes, such as BRCA1 or BRCA2 carriers, display pronounced cancer predisposition, attesting to the powerful role such genes play in cancer avoidance. In evaluating the impact of the low levels of exposure to IR to which individuals might be exposed, it is thus vital to critically assess the efficiency of the DDR processes to protect against low levels of DNA damage. Despite this, the vast majority of studies aimed at a mechanistic understanding of the DDR processes have utilized high doses of DNA damaging agents. In this report, an attempt is made to exploit our current understanding of the DDR mechanisms gained largely from the use of high dose exposure, to consider the likely impact of low dose/dose rate exposure to IR. This raises the issue of what dose/dose rate exposure is of physiological relevance. Although for the most part, my discussion does not pertain to a

precise definition, I have generally considered a low dose to be < 100 mg and a low dose rate to provide an accumulative dose of < 100 mg Gy.

Endogenous versus radiation induced DNA damage

Cellular DNA is constantly subjected to an onslaught of endogenous DNA damage, with estimates suggesting that greater than fifty thousand lesions arise per day in each cell (Lindahl 1993). The damage to DNA includes single strand breaks (SSBs), depurination and depyrimidations, alkylation damage, oxidative lesions, deaminations and double strand breaks (DSBs). Although by far the least frequent lesion induced, a DSB is arguably the most biologically significant since, if unrepaired it can cause cell death and, perhaps more importantly, if misrepaired, it can result in genetic rearrangements, a step in the etiology of carcinogenesis. The focus here will rest on the impact of DSBs since a vast amount of literature has pointed to a DSB being the most biologically significant, radiation-induced lesion. It follows from the above that low dose/dose rate exposure induces DSBs that are additive to an endogenous background level of DSBs. Two issues are important in considering the impact of this; (1) what is the level of endogenous DSB formation? and (2) do radiation induced DSBs differ from endogenously arising DSBs and what is the significance of any difference? There is little information to accurately assess endogenous DSB levels. However, DSBs do arise since cell lines lacking the ability to rejoin DSBs (e.g., DNA ligase IV or XRCC4 null cell lines) are embryonic lethal and fail to replicate in culture unless also defective in p53. It has been estimated that a cell incurs twenty thousand SSBs per day from oxidative damage (Lindahl 1993). Oxidative damage induces approximately one DSB per two thousand SSBs; hence one might expect 10-20 DSBs to arise per cell per day. Furthermore, DSBs can potentially arise when a transcriptional or replication complex

encounters base damage or an SSB. DSBs generated by oxidative damage can arise as two overlapping SSBs whilst a DSB generated when an SSB encounters the transcription or replication machinery may not have overlapping single strand tails. DSBs generated by either of these routes will likely have damaged termini. Since all known mammalian ligases require 3'OH and 5' P termini for ligation, these damaged ends will require processing prior to ligation. Cells, however, are fully equipped with mechanisms to process such lesions (Barnes and Lindahl 2004). Radiation-induced damage may differ, however. Depending upon the linear energy transfer (LET) of the radiation, there are likely to be multiple lesions, including SSBs, base damages and DSBs, in close proximity. Such lesions have been termed complex lesions, in distinction to “dirty ended lesions” generated by oxidative damage.

Repair of endogenous versus radiation induced lesions.

It is important to understand how the complexity of a lesion influences its repair. The available evidence suggests that the majority of DSBs, including those induced by high LET radiation (e.g., particle radiation), can be rejoined by the cellular machinery (for example DSB repair studies in (Kuhne et al. 2000; Riballo et al. 2004). In the ensuing discussion, high and low LET radiations will generally be taken to represent particle and photon radiations, respectively. The critical issue is whether such DSBs can be repaired accurately. One important consideration is whether coding information lost at the DSB can be accurately regenerated. This will be further discussed below as part of the DSB repair pathways.

Modelling studies of radiation damage suggest that both high, and to a lesser extent, low LET radiation generate complex DNA lesions at which coding information will be lost either as a consequence of damaged bases arising at the same position in both strands or from sequences being lost from both strands (Nikjoo et al. 1997; Nikjoo et al. 1999; Nikjoo et al. 2000; Nikjoo et al. 2001; Nikjoo et al. 2002). Although this can also likely arise from endogenous

damage, the higher complexity of radiation induced DSBs likely results in a higher frequency of lesions associated with loss of coding information (Fig. 1). This is a critical issue in assessing the fidelity of DSB repair. A second importation consideration is whether DSBs that arise in close proximity but on distinct chromosomes have the potential to rejoin to each other (i.e., to misrejoin), thereby generating genomic rearrangements (Fig. 1). This may occur frequently following high LET radiation. Since a cell may harbour endogenous DSBs at any given time, even low dose/dose rate exposure inducing not more than a single DSB/cell may have the potential to enhance genomic rearrangement events. Further, the slower rate of repair of complex DSBs compared with less complex DSBs may also impact upon the fidelity of repair: slow repair may provide the opportunity for end degradation and a greater probability for a radiation induced DSB to encounter a closely located endogenous break.

It is perhaps also important to consider the impact of DNA damage that can create a DSB upon replication. As mentioned above endogenous damage induced by reactive oxygen species generates predominantly base damage and hence SSB formation. Such damage is efficiently and rapidly repaired and there is only a small window allowing such damage to be present at a replication fork. Radiation, however, can also create non-DSB clustered damage, which may be more slowly repaired than the single lesions induced by ROS. Thus, there may be a greater chance of non-DSB clustered damage being encountered at a replication fork. Further, the complexity of such damage may enhance the likelihood of DSB generation at a collapsed replication fork.

DNA damage response mechanisms.

Cells exploit two damage response strategies to limit the impact of DSBs; the damage can be repaired using DSB repair pathways and/or can initiate signal transduction pathways that raise the alert to the presence of DSBs (Fig. 2). The signal transduction process can

activate a range of mechanisms of which the most significant are cell cycle checkpoint arrest and apoptosis. The signalling process also influences DSB repair although most DSB repair occurs independently of the DSB signalling response. First an overview the DSB repair processes will be presented, focusing particularly on aspects important for evaluating their ability to accurately repair low levels of DSBs induced either endogenously or by ionizing radiation.

A. Mechanisms of DSB repair:

- Core NHEJ.

The major pathway that repairs radiation induced DSBs is DNA non-homologous end-joining (NHEJ) (for reviews see Hefferin and Tomkinson 2005; Jeggo and Lobrich 2006b; Wyman and Kanaar 2006; van Gent and van der Burg 2007). The first step of NHEJ is the binding of a heterodimeric protein, Ku, to double stranded (ds) DNA ends. Ku is a basket shaped molecule with a central hole of sufficient diameter to allow the threading of dsDNA (Walker et al. 2001). Furthermore, this structure endows Ku with the ability to translocate along the DNA (de Vries et al. 1989, Blier et al. 1993, Smith and Jackson 1999). The presence of Ku on the DNA end inhibits exonucleolytic digestion, thereby helping to protect the end (Liang and Jasin 1996). When DNA bound, Ku recruits the large catalytic subunit of the DNA dependent protein kinase complex, termed DNA-PKcs, thereby generating the intact DNA-PK complex (Dvir et al. 1992, Gottlieb and Jackson 1993). The precise function of the DNA-PK complex is still unknown but increasing evidence points to it having a regulatory function in NHEJ. Most importantly, DNA-PK appears to regulate processing of the DNA ends to generate the 3'OH and 5'P ends needed for ligation (Cui et al. 2005; Meek et al. 2007). Finally, a complex of DNA ligase IV, XRCC4 and

XLF promotes the rejoining step. Strong data has shown that NHEJ is the major DSB rejoining pathway in G0, G1 and G2 and indirect evidence suggests that it also has an important role in S phase (Fig. 1, 2 and 3) (Riballo et al. 2004; Deckbar et al. 2007; Krempler et al. 2007). Consistent with this, cells lacking NHEJ are exquisitely radiation sensitive (Jeggo 1990). NHEJ effectively rejoins DNA ends without using any significant homology and, most importantly, without the use of an undamaged template. Thus, it is difficult to see how any sequence information lost of the break site can be accurately reconstituted. NHEJ is, thus, often referred to as an error prone repair mechanism but this phraseology is misleading. NHEJ has the potential to rejoin DSBs accurately provided bases are not lost or damaged at the same site on both strands. Whilst most DSBs induced by high LET IR may be associated with loss of coding information, it is likely that this is not the case for most endogenously generated DSBs, even though they may frequently harbour damaged termini. Our current understanding of how broken ends remain tethered during NHEJ is still sparse. However, it is likely that NHEJ will have the potential to cause translocations by rejoining DSB ends generated in close proximity (Fig. 1).

- **Homologous Recombination (HR)**

HR represents a second DSB rejoining process (for reviews see Johnson and Jasin 2001; West 2003; Thorslund and West 2007). Whereas NHEJ uses little or no homology to effect rejoining, HR is an elegant process that effectively uses a homologous chromosome as a template for rejoining. In brief, the process involves the generation of single stranded DNA by 5' to 3' resection at the DSB, which becomes coated by RPA, a single stranded DNA binding protein. Subsequently, Rad51, aided by BRCA2, replaces RPA and promotes invasion of the intact

homologous DNA strand. The displaced strand can form a “D-loop” to act as a template for repair of the other broken strand. Hence, new DNA can be synthesized using the invading DNA as a template, and finally resolution of the “heteroduplex” molecule occurs followed by ligation of the DNA ends. The process is complex but has the potential to allow for large repair tracts and hence the ability to recover coding information lost at the site of the DSB. Although HR represents the major mechanism for DSB repair in lower organisms and yeast mutants lacking HR are extremely radiosensitive, it has a less significant impact in mammalian cells. One reason for this is that in mammalian cells, HR only functions during late S/G2 phase when a sister chromatid is available as the source of homology (Johnson and Jasin 2000). Indeed, homologous chromosomes are rarely used for HR in mammalian cells. Although it has been argued that HR functions to repair the majority of DSBs in G2 phase, in contrast to NHEJ, which functions in G1, emerging evidence suggests that HR only has a modest impact on DSB rejoining even in G2, rejoining at most 20 % of x- or γ -ray induced DSBs (Wu et al. 2008) (Beucher, manuscript submitted) (Fig. 3). Indeed, the major role of HR appears to be to repair one-sided DSBs that arise when a replication fork encounters a lesion that blocks replication. In line with this, HR defective cell lines show only modest radiation sensitivity whilst NHEJ defective mutants are dramatically radiation sensitive in all cell-cycle phases (Jeggo 1990, Thacker and Zdzienicka 2003). The use of an undamaged template to reconstitute genetic information lost at the break site provides HR with the ability to repair even complex DSBs accurately. It is, thus, curious that despite this, HR is used infrequently to repair DSBs in G2 phase, when such an undamaged template is available. This may reflect the fact that endogenously generated DSBs are not highly complex and hence there has not been selective pressure to optimize selection of the repair processes.

Additionally, DSBs repaired by HR can lead to genomic rearrangements (Fig. 1) (Weinstock et al. 2006). Hence, it appears possible that HR can undergo template switching providing a route for misrejoining. It should also be appreciated that HR can also potentially cause base changes following synthesis at persistent, miscoding base damage.

- **Back-up NHEJ (B-NHEJ)**

B-NHEJ has been described as a further DSB repair pathway that involves PARP and XRCC1, two proteins that function in single strand break repair (Fig. 3) (Perrault et al. 2004; Wang et al. 2006; Windhofer et al. 2007; Wu et al. 2008). However, B-NHEJ is effectively a process that functions opportunistically when NHEJ does not function due to a lack of one of the core NHEJ proteins. Since this happens rarely in human patients, it will not be considered in detail here. It is worth noting, however, that B-NHEJ likely exploits microhomology at the DSB junction, effecting rejoining by limited resection at the break sites, pairing of the single stranded regions that harbour microhomologies followed by two single strand repair events. It, therefore, represents a low fidelity rejoining mechanism.

- **A sub-component of NHEJ that rejoins the slow component of DSBs.**

Classical studies on DSB rejoining using a range of techniques have demonstrated that cells rejoin DSBs with biphasic kinetics with approximately 80 % of the DSBs being rejoined with fast kinetics whilst a smaller fraction are rejoined with much slower kinetics (DiBiase et al. 2000). Interestingly, it was recently demonstrated that in G0/G1 phase the fast component of DSB requires the core NHEJ proteins whilst a 10-20 % sub-fraction of X- or γ -ray induced DSBs requires additional proteins as well

as the core NHEJ proteins (Riballo et al. 2004). These additional proteins include ataxia telangiectasia mutated (ATM), the nuclease Artemis, the mediator protein, 53BP1, and γ -H2AX. These additional proteins appear to be required uniquely for the slow component of DSB repair. Interestingly, the fraction of DSBs repaired in an ATM/Artemis dependent manner appears to relate, at least partly, to the complexity of the DNA damage, raising the possibility the cells might possess a specific end-processing pathway to repair “complex” DSBs or lesions with dirty ends. However, more recently studies have suggested that although end complexity may represent an aspect of the story, the requirement for additional factors never exceeds 25 % of the induced DSBs even for radiation qualities which generate highly complex DSBs and for chemical agents that induce a homogenous class of DSBs, the value is similar to the 10 % fraction observed after x- or γ rays (Goodarzi et al, 2008). Instead, it has been suggested that the additional factors are required to facilitate the repair of DSBs located at specific DNA regions such as those in heterochromatic DNA. Thus, whilst this aspect of DSB repair is important to consider, there is currently little direct evidence that cells have a specific pathway to facilitate the repair of highly complex DSB lesions.

In summary, NHEJ represents the major DSB rejoining pathway in G0, G1 and G2, and likely rejoins most DSBs that are not directly replication associated during S phase. Therefore, the critical issue is the accuracy with which NHEJ rejoins radiation induced DSBs (Fig. 1). Although cells have an impressive ability to remove damaged termini and generate ends suitable for ligation, our current understanding of the process makes it difficult to see how NHEJ can accurately regenerate sequence information that might be lost at a DSB site. Surprisingly, although HR represents an elegant mechanism to repair the highly complex

DSBs induced by IR, and particularly high LET IR, there is little evidence that it plays a major role in repairing such DSBs. One possibility that requires further examination is whether HR plays a greater role in the repair of DSBs induced by high LET radiation. Since HR appears to function in G2 to repair the slow component of DSB repair, and since high LET-induced DSBs are repaired more slowly, it is possible that HR will contribute to a higher percentage of DSB repair (Lobrich, unpublished findings)[†]. Nonetheless, the current evidence suggests that NHEJ remains the major DSB repair process even in G2 phase. Finally, a critical issue for carcinogenesis is likely to be a translocation event which can arise when two previously unconnected DNA ends are rejoined. Our current knowledge, would suggest that both NHEJ and HR have the potential to cause translocation events (Fig. 2).

Repair of high LET DNA DSBs.

In the section above, an evaluation was performed whether the known DSB repair mechanisms are likely to be able to repair complex DSBs accurately. Studies to assess DSB repair of x-ray, γ ray or alpha particle irradiation using pulsed field gel electrophoresis (PFGE) and, more recently, the enumeration of centres of DSB repair (called γ -H2AX foci), which can be visualized by microscopy, have provided strong evidence that DSBs generated following exposure to all these forms of radiation can be repaired albeit with differing kinetics (Kuhne et al. 2000; Riballo et al. 2004). Interestingly, cells lacking the NHEJ protein, Ku, (Xrs cells) show a markedly reduced ability to repair DSBs induced by x and γ rays and are highly sensitive to both forms of radiation compared to control cells (Kemp et al. 1984; Jeggo 1990). They are also impaired in the rejoining of DSBs induced by alpha particle irradiation compared to control cells, yet their level of survival is only slightly reduced compared to control cells (Thacker and Stretch 1985; Hill et al. 2004; Riballo et al. 2004).

[†]

This strongly suggests that although the complex DSBs induced by x-rays and by alpha particle irradiation are repaired by NHEJ, the rejoining of alpha particle induced DSBs, which may involve multiple DSBs in close proximity, imparts less benefit on survival. This, in turn, strongly suggests that rejoining of the complex DSBs induced by such irradiation might frequently be misrepaired, generating either sequence loss or rearrangements, which may arise due to the close proximity of multiple DSBs (Fig. 1). This implies further that, at least a reasonable fraction of the DSBs induced by x- or γ rays are repaired correctly, or any small deletions generated are tolerated.

Damage Response Signalling.

Cells exploit two major signal transduction pathways in response to DNA damage (Shiloh 2003; Abraham 2004; Kurz and Lees-Miller 2004). These represent overlapping pathways each activated by a distinct but related phosphoinositol 3-kinase like kinase (PIKK). Ataxia telangiectasia mutated (ATM) is a PIKK activated by DSBs and hence represents the most significant kinase activating signalling following radiation exposure (Kurz and Lees-Miller 2004). AT and Rad3-related (ATR) is a related kinase activated by single stranded regions of DNA, which can occur following the stalling of replication forks or during the processing of bulky lesions such as a pyrimidine dimer (Cortez et al. 2001; Zou et al. 2002; Zou and Elledge 2003; Zou et al. 2003). ATR can be activated by IR in S phase cells if the induced lesions cause a replication blockage. ATM and ATR share overlapping substrates although substrates specific for one or the other kinase have been described. Activation of ATM or ATR can result in cell cycle checkpoint arrest and/or apoptosis (Abraham 2001). ATM activation can also influence the DNA repair process and likely also impacts upon chromatin structure (Riballo et al. 2004; Ziv et al. 2006). The requirement of ATM signalling for a component of DSB repair has been discussed above. This section will focus on the

process of cell cycle checkpoint arrest and its relevance for considering the impact of low dose/dose rate exposure to IR

Cell cycle checkpoint arrest occurs at defined positions in the cell cycle, which include the transition between G1 and S phase (G1/S checkpoint), entry into mitosis (the G2/M checkpoint) and during S phase (the intra-S phase checkpoint) (Elledge 1996, Caspari and Carr 2002). One important function of these checkpoints is to prevent cells progressing through important steps in DNA metabolism, such as replication or mitosis, in the presence of DNA damage. Hence the cell is provided with additional time for repair (Deckbar et al. 2007). At least one of these checkpoints in mammalian cells, the G1/S checkpoint, can also serve to permanently prevent the proliferation of damaged cells, thus providing an alternative to apoptosis (Linke et al. 1997). Given that ATM, as described above, is required for the repair of DSBs that are rejoined with slow kinetics, checkpoint arrest provides “added value” of ATM signalling allowing additional time to repair those DSBs for which it is uniquely required (Lobrich and Jeggo 2005). Attempts to replicate or perform mitosis and cytokinesis in the presence of unrepaired DSBs will very likely result in loss of genomic material or elevated misrepair. Hence cell cycle checkpoint arrest likely represents a critical damage response mechanism that is important for the maintenance of genomic stability, although it may be less important for survival (Lobrich and Jeggo 2005, Jeggo and Lobrich 2006a; Lobrich and Jeggo 2007).

An important aspect in considering the impact of low doses/dose rates of IR is the sensitivity of the signal transduction process in detecting DNA damage and signalling to the cell cycle checkpoint machinery. Based on studies in yeast and the sensitivity of activation of ATM signalling, it was anticipated that the checkpoint machinery would be activated by a single DSB and maintain arrest until the completion of DSB repair. However, recent studies have demonstrated that doses of IR which introduce less than 10-20 DSBs/cell fail to activate

G2/M checkpoint arrest and that, when arrest is initiated following exposure to higher IR doses, it is not maintained until the completion of repair but is released when 10-20 DSBs/cell remain (Deckbar et al. 2007). Indeed, chromosome breaks can be observed in cells released from checkpoint arrest and, in fact, the majority of chromosome breaks arise via this route rather than in cells that escape checkpoint arrest. Low dose hypersensitivity is a phenomenon in which cells appear to be relatively more sensitive to very low doses of IR (< 0.3 Gy) compared to slightly higher doses, generating a dip in the survival curve at doses of 0.1-0.3 Gy (Marples et al. 2004). Evidence has suggested that this can be attributed to the G2 population of cells and it is highly likely that this sensitivity can be attributed to a failure to activate G2/M checkpoint arrest at low doses (Short et al. 2003). This lack of sensitivity of the G2/M checkpoint implies firstly that low doses of radiation have a finite possibility of inducing chromosome breakage and/or causing cell death. Secondly, exposure to doses that activate checkpoint arrest will also fail to fully protect cells from entering mitosis with chromosome breaks.

To date the sensitivity of the G1/S checkpoint has not been accurately determined. The available evidence suggests that two distinct G1/S checkpoints may exist of which one, a p53 dependent process, may have greater sensitivity than the G2/M checkpoint, and may indeed be sensitive to a single DSB (Huang et al. 1996). This critical question needs to be addressed applying the more sensitive monitors of DSB induction currently available.

Apoptosis represents a distinct process to checkpoint arrest to prevent the proliferation of cells with DNA damage. The activation of apoptosis appears to be highly cell type dependent with some cells, such as skin fibroblasts, rarely undergoing apoptosis in response to IR, whilst others, such as cells of lymphoid origin, readily exploit apoptosis to remove damaged cells. Currently, there is little data available to assess the sensitivity of the

apoptotic machinery to DSB induction although it is likely that sensitivity will be highly tissue dependent.

In summary, cell cycle checkpoint arrest represents an important aspect of the response to DNA damage, which has a major impact on the maintenance of genomic stability by providing additional time for DSB repair prior to progression through critical metabolic steps such as replication or mitosis. It appears to have a lesser impact on survival. Surprisingly, the G2/M checkpoint is relatively insensitive and has a threshold of 10-20 DSBs (Deckbar et al. 2007). Further, low doses of IR fail to activate G2/M arrest. Strikingly, cells appear able to progress through mitosis with DSBs and enter the subsequent G1 phase (Lobrich, unpublished observations). If acentric fragments are lost, then the probability of accurate DSB repair will be much diminished. Nonetheless, such DSBs may well be misrepaired in G1, thereby providing a window by which genomic rearrangements or loss of genetic material can occur.

Damage response mechanisms functioning in stem cells.

Important recent evidence has demonstrated the stem cell origin of many cancer cells (Lee and Herlyn 2007). The importance of the maintenance of genomic stability for stem cells has been considered previously and one route by which this may be achieved is by a non replicated master strand being maintained by a process of asymmetric division (Cairns 2006). Whilst such a mechanism will serve to prevent the propagation of sequence errors generated during replication, it will not prevent the impact of DNA damage incurred from agents such as radiation. For this reason, it has been proposed that stem cells might prefer to die following DNA damage rather than attempt repair and, consequently that their repair processes might be down regulated (Hong et al. 2007). This raises the important question as to how the DSB repair processes function in stem cells. Some stem cells appear to be extremely sensitive to

IR raising the possibility that they might indeed undergo apoptosis rather than risk erroneous repair. Thus, it is important to assess the operation of the damage responses processes in stem cells by evaluating which DSB repair pathways are functional, the threshold of the cell cycle checkpoints and sensitivity of induction of apoptosis. For mammalian cells, it is highly likely that altering the threshold for apoptosis provides an efficient means to remove cells that have incurred damage.

Conclusions and future questions to be addressed.

Our current understanding of the mechanisms of DSB repair indicate that NHEJ, the predominant mechanism that rejoins radiation induced DSBs, is unlikely to be able to accurately restore the genomic sequence at DSBs in which coding information is lost. This may well represent a high percentage of DSBs induced by high LET radiation. Given the spectrum of lesions induced by x- and γ rays, complex DSBs are also predicted to be induced at a reasonable frequency by low LET radiation. HR, in contrast, since it exploits an undamaged template to repair DSBs, has the potential to achieve a higher level of fidelity. Curiously, however, HR only functions in late S and G2 phase and even in G2 phase, only accounts for a low percentage (maybe 10 to 20 %) of DSB repair. Another route by which erroneous DSB repair can occur is via the joining of incorrect DSB ends generating genomic rearrangements. The occurrence of several correlated DSBs in close proximity along the path of a single high LET radiation particle makes this a prominent mechanism for this type of radiation. Moreover, even x- and γ ray induced DSBs, occurring as single DSBs after low dose/dose rate exposure, will have the potential to interact with endogenously induced DSBs and thus are likely to enhance the level of rearrangements. It is likely that both the HR and NHEJ machinery have the potential to generate rearrangements by this route. Collectively, based on our current knowledge of the DSB repair mechanisms, I would suggest that a

mammalian cell will be unable to ensure faithful repair of all DSBs induced by even low doses of IR.

Two other processes that are important in the maintenance of genomic stability are cell cycle checkpoint arrest and apoptosis. Current evidence indicates that the G2/M checkpoint allows the generation of chromosome breakage and, indeed, that most chromosome breaks arise from cells released from checkpoint arrest. Such cells can traverse mitosis and enter G1 with chromosome DSBs, but having lost any acentric fragments generated. There is abundant evidence suggesting that cells possess mechanisms to heal or repair such DSBs, including telomere fusion events. This may eliminate the presence of a DSB allowing such cells, which may well harbour translocations or rearrangements, to propagate. Although the sensitivity of the G1/S checkpoint has not been fully assessed, the fact that chromosome breaks arise in Artemis deficient cells following irradiation in G1 phase, provides strong evidence that the G1/S checkpoint fails to permanently arrest cells with low levels of DSBs. In conclusion, our current understanding of the process of cell cycle checkpoint arrest suggests that the G2 phase of the mammalian cell cycle may represent a particularly sensitive window for the generation of rearrangements. Clearly, it is critical to evaluate the level of fidelity achieved during the repair of low LET induced DSBs as well as to assess the sensitivity of the G1/S checkpoint. Recent studies on additional endpoints activated by ATM signalling have raised the possibility that further events need to be evaluated. There is mounting evidence that an important aspect of ATM signalling is to alter the chromatin structure of DNA in the vicinity of the DSB (Ziv et al. 2006). There is evidence that DSBs induced within heterochromatic DNA are particularly difficult to repair and may require some “opening” of heterochromatic DNA. Since DNA compaction is one factor that inhibits transcription, it is important to assess how much “opening” of compacted

DNA occurs after radiation damage and whether this can result in normally inactive genes being erroneously activated.

In summary, an understanding of the damage response mechanisms has provided important insight that is informative in evaluating the likely impact of radiation damage. Without any consideration of such phenomena as the bystander effect or delayed genetic instability, the current knowledge of the damage response mechanisms suggests that low levels of radiation have the potential to cause genetic changes that underlie cancer induction, and thus even a low level of radiation exposure has the potential to cause cancer.

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FOOTNOTES

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References.

- Abraham RT. Cell cycle checkpoint signaling through the ATM and ATR kinases. *Genes Dev* 15: 2177–2196.; 2001.
- Abraham RT. PI 3-kinase related kinases: 'big' players in stress-induced signaling pathways. *DNA Repair (Amst)* 3: 883–887; 2004.
- Barnes DE, Lindahl T. Repair and genetic consequences of endogenous DNA base damage in mammalian cells. *Annu Rev Genet* 38: 445–476; 2004.
- Blier PR, Griffith AJ, Craft J, Hardin JA. Binding of Ku protein to DNA. Measurement of affinity for ends and demonstration of binding to nicks. *Journal of Biological Chemistry* 268: 7594–7601; 1993.
- Cairns J. Cancer and the immortal strand hypothesis. *Genetics* 174: 1069–1072; 2006.
- Caspari T, Carr AM. Checkpoints: how to flag up double-strand breaks. *Curr Biol.* 12: R105–R107; 2002.
- Cortez D, Guntuku S, Qin J, Elledge SJ. ATR and ATRIP: partners in checkpoint signaling. *Science* 294: 1713–1716.; 2001.
- Cui X, Yu Y, Gupta S, Cho YM, Lees-Miller SP, Meek K. Autophosphorylation of DNA-dependent protein kinase regulates DNA end processing and may also alter double-strand break repair pathway choice. *Mol Cell Biol* 25: 10842–10852; 2005.
- de Vries E, van Driel W, Bergsma WG, Arnberg AC, van der Vliet PC. HeLa nuclear protein recognizing DNA termini and translocating on DNA forming a regular DNA-multimeric protein complex. *Journal of Molecular Biology* 208: 65–78; 1989.
- Deckbar D, Birraux J, Krempler A, Tchouandong L, Beucher A, Walker S, Stiff T, Jeggo PA, Lobrich M. Chromosome breakage after G2 checkpoint release. *Journal of Cell Biology* 176: 748–755; 2007.

- DiBiase SJ, Zeng ZC, Chen R, Hyslop T, Curran WJ, Jr., Iliakis G. DNA-dependent protein kinase stimulates an independently active, nonhomologous, end-joining apparatus. *Cancer Res* 60: 1245–1253; 2000.
- Dvir A, Peterson SR, Knuth MW, Lu H, Dynan WS. Ku autoantigen is the regulatory component of a template-associated protein kinase that phosphorylates RNA polymerase II. *Proceedings of the National Academy of Sciences of the United States of America* 89: 11920–11924; 1992.
- Elledge SJ. Cell cycle checkpoints: preventing an identity crisis. *Science* 274: 1664-1672; 1996.
- Goodarzi AA, Noon AT, Deckbar D, Ziv Y, Shiloh Y, Lobrich M, Jeggo PA. ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. *Mol. Cell* 31: 167-177; 2009
- Gottlieb TM, Jackson SP. The DNA-dependent protein kinase: requirement of DNA ends and association with Ku Antigen. *Cell* 72: 131–142; 1993.
- Hefferin ML, Tomkinson AE. Mechanism of DNA double-strand break repair by non-homologous end joining. *DNA Repair (Amst)* 4: 639–648; 2005.
- Hill MA, Herdman MT, Stevens DL, Jones NJ, Thacker J, Goodhead DT. Relative sensitivities of repair-deficient mammalian cells for clonogenic survival after alpha-particle irradiation. *Radiat Res* 162: 667–76; 2004.
- Hong Y, Cervantes RB, Tichy E, Tischfield JA, Stambrook PJ. Protecting genomic integrity in somatic cells and embryonic stem cells. *Mutat Res* 614: 48–55; 2007.
- Huang LC, Clarkin KC, Wahl GM. Sensitivity and selectivity of the DNA damage sensor responsible for activating p53-dependent G1 arrest. *Proc Natl Acad Sci U S A* 93: 4827–4832; 1996.

- Jeggo PA. Studies on mammalian mutants defective in rejoining double-strand breaks in DNA. *Mutat Res* 239: 1–16; 1990.
- Jeggo PA, Lobrich M. Contribution of DNA repair and cell cycle checkpoint arrest to the maintenance of genomic stability. *DNA Repair (Amst)* 5: 1192–1198; 2006a.
- Jeggo PA, Lobrich M. Radiation induced DNA damage responses. *Radiation Protection Dosimetry* 122: 124–127; 2006b.
- Johnson RD, Jasin M. Sister chromatid gene conversion is a prominent double-strand break repair pathway in mammalian cells. *Embo J* 19: 3398–3407.; 2000.
- Johnson RD, Jasin M. Double-strand-break-induced homologous recombination in mammalian cells. *Biochem Soc Trans* 29: 196–201.; 2001.
- Kemp LM, Sedgwick SG, Jeggo PA. X-ray sensitive mutants of Chinese hamster ovary cells defective in double-strand break rejoining. *Mutat Res* 132: 189–196; 1984.
- Krempler A, Deckbar D, Jeggo PA, Lobrich M. An Imperfect G(2)/M Checkpoint Contributes to Chromosome Instability Following Irradiation of S and G(2) Phase Cells. *Cell Cycle* 6: 1682–1686; 2007.
- Kuhne M, Rothkamm K, Lobrich M. No dose-dependence of DNA double-strand break misrejoining following alpha-particle irradiation. *Int J Radiat Biol* 76: 891–900.; 2000.
- Kurz EU, Lees-Miller SP. DNA damage-induced activation of ATM and ATM-dependent signaling pathways. *DNA Repair (Amst)* 3: 889–900; 2004.
- Lee JT, Herlyn M. Old disease, new culprit: tumor stem cells in cancer. *J Cell Physiol* 213: 603–609; 2007.
- Liang F, Jasin M. Ku80-deficient cells exhibit excess degradation of extrachromosomal DNA. *Journal of Biological Chemistry* 271: 14405–14411; 1996.
- Lindahl T. Instability and decay of the primary structure of DNA. *Nature* 362: 709–714; 1993.

- Linke SP, Clarkin KC, Wahl GM. p53 mediates permanent arrest over multiple cell cycles in response to gamma-irradiation. *Cancer Research* 57: 1171–1179; 1997.
- Lobrich M, Jeggo PA. Harmonising the response to DSBs: a new string in the ATM bow. *DNA Repair (Amst)* 4: 749–759; 2005.
- Lobrich M, Jeggo PA. The two edges of the ATM sword: Co-operation between repair and checkpoint functions. *Radiother Oncol* 76: 112–118; 2005.
- Lobrich M, Jeggo PA. The impact of a negligent G2/M checkpoint on genomic instability and cancer induction. *Nat Rev Cancer* 7: 861–869; 2007.
- Marples B, Wouters BG, Collis SJ, Chalmers AJ, Joiner MC. Low-dose hyper-radiosensitivity: a consequence of ineffective cell cycle arrest of radiation-damaged G2-phase cells. *Radiat Res* 161: 247–255; 2004.
- Meek K, Douglas P, Cui X, Ding Q, Lees-Miller SP. trans Autophosphorylation at DNA-dependent protein kinase's two major autophosphorylation site clusters facilitates end processing but not end joining. *Mol Cell Biol* 27: 3881–3890; 2007.
- Nikjoo H, Bolton CE, Watanabe R, Terrissol M, O'Neill P, Goodhead DT. Modelling of DNA damage induced by energetic electrons (100 eV to 100 keV). *Radiat Prot Dosimetry* 99: 77–80.; 2002.
- Nikjoo H, Munson RJ, Bridges BA. RBE-LET relationships in mutagenesis by ionizing radiation. *Radiation Research* 40 Suppl.: 85–105; 2000.
- Nikjoo H, O'Neill P, Goodhead DT, Terrissol M. Computational modelling of low-energy electron-induced DNA damage by early physical and chemical events. *Int J Radiat Biol* 71: 467–483; 1997.
- Nikjoo H, O'Neill P, Terrissol M, Goodhead DT. Quantitative modelling of DNA damage using Monte Carlo track structure method. *Radiat Environ Biophys* 38: 31–38; 1999.

- Nikjoo H, O'Neill P, Wilson WE, Goodhead DT. Computational approach for determining the spectrum of DNA damage induced by ionizing radiation. *Radiat Res* 156: 577–583.; 2001.
- Perrault R, Wang H, Wang M, Rosidi B, Iliakis G. Backup pathways of NHEJ are suppressed by DNA-PK. *J Cell Biochem* 92: 781–794; 2004.
- Riballo E, Kuhne M, Rief N, Doherty A, Smith GC, Recio MJ, Reis C, Dahm K, Fricke A, Krempler A, Parker AR, Jackson SP, Gennery A, Jeggo PA, Lobrich M. A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci. *Mol Cell* 16: 715–724; 2004.
- Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. *Nat Rev Cancer* 3: 155–168; 2003.
- Short SC, Woodcock M, Marples B, Joiner MC. Effects of cell cycle phase on low-dose hyper-radiosensitivity. *Int J Radiat Biol* 79: 99–105; 2003.
- Smith GC, Jackson SP. The DNA-dependent protein kinase. *Genes Dev.* 13: 916–934; 1999.
- Thacker J, Stretch A. Responses of 4 X-ray-sensitive CHO cell mutants to different radiation and to irradiation conditions promoting cellular recovery. *Mutation Research* 146: 99–108; 1985.
- Thacker J, Zdzienicka MZ. The mammalian XRCC genes: their roles in DNA repair and genetic stability. *DNA Repair (Amst)* 2: 655–672; 2003.
- Thorslund T, West SC. BRCA2: a universal recombinase regulator. *Oncogene* 26: 7720–7730; 2007.
- van Gent DC, van der Burg M. Non-homologous end-joining, a sticky affair. *Oncogene* 26: 7731–7740; 2007.
- Walker JR, Corpina RA, Goldberg J. Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. *Nature* 412: 607–614.; 2001.

- Wang M, Wu W, Rosidi B, Zhang L, Wang H, Iliakis G. PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways. *Nucleic Acids Res* 34: 6170–6182; 2006.
- Weinstock DM, Richardson CA, Elliott B, Jasin M. Modeling oncogenic translocations: distinct roles for double-strand break repair pathways in translocation formation in mammalian cells. *DNA Repair (Amst)* 5: 1065–1074; 2006.
- West SC. Molecular views of recombination proteins and their control. *Nat Rev Mol Cell Biol* 4: 435–445.; 2003.
- Windhofer F, Wu W, Wang M, Singh SK, Saha J, Rosidi B, Iliakis G. Marked dependence on growth state of backup pathways of NHEJ. *Int J Radiat Oncol Biol Phys* 68: 1462–1470; 2007.
- Wu W, Wang M, Singh SK, Mussfeldt T, Iliakis G. Repair of radiation induced DNA double strand breaks by backup NHEJ is enhanced in G2. *DNA Repair (Amst)* 7: 329–338; 2008.
- Wyman C, Kanaar R. DNA double-strand break repair: all's well that ends well. *Annu Rev Genet* 40: 363–383; 2006.
- Ziv Y, Bielopolski D, Galanty Y, Lukas C, Taya Y, Schultz DC, Lukas J, Bekker-Jensen S, Bartek J, Shiloh Y. Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. *Nat Cell Biol* 8: 870–876; 2006.
- Zou L, Cortez D, Elledge SJ. Regulation of ATR substrate selection by Rad17-dependent loading of Rad9 complexes onto chromatin. *Genes Dev.* 16: 198–208; 2002.
- Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* 300: 1542–1548.; 2003.

Zou L, Liu D, Elledge SJ. Replication protein A-mediated recruitment and activation of Rad17 complexes. *Proc Natl Acad Sci U S A* 100: 13827–13832. Epub 2003 Nov 6; 2003.

Figure Legends.

Figure 1. Misrepair events that can be generated following exposure to IR.

Ionizing radiation can result in damaged bases at the same site in both strands or multiple lesions in close proximity on one DNA molecule. Both situations have the potential to result in loss of coding information. NHEJ will be unable to reconstitute any sequence information lost in this way whilst HR can restore the original DNA sequence by using an undamaged sister homologue as a template for resynthesis. Translocations can also arise when DSBs are generated in close proximity of distinct chromosomes. Both HR and NHEJ appear able to generate translocations via this mechanism.

Figure 2. Features of the damage response mechanisms important for the maintenance of genomic stability in response to low doses/dose rates.

DNA damage response mechanisms that function in response to DSBs encompass pathways of DSB repair and signal transduction pathways. HR and NHEJ represent the major DSB repair pathways and ATM signalling is activated by DSBs. The fidelity by which the DSB repair pathways rejoin DSBs is a critical issue for considering low dose exposure. This includes whether sequences lost at the junction can be recovered and whether translocations are generated if two erroneous DNA ends are rejoined. ATM signalling activates cell cycle checkpoint arrest and apoptosis. The critical issue here is the sensitivity of these processes. Current evidence suggests that the G2/M checkpoint has a sensitivity threshold of 10-20 DSBs and that chromosome breakage can arise in cells released from checkpoint arrest.

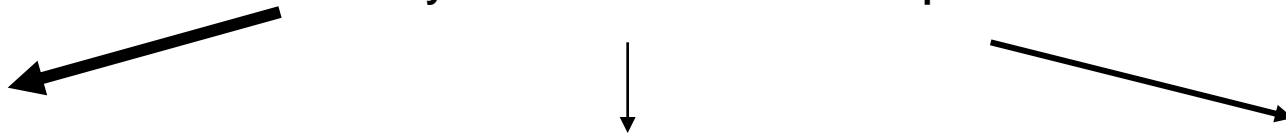
Figure 3. Pathways of double strand break repair.

NHEJ represents the most significant DSB repair pathway for rejoining radiation induced DSBs. HR represents another important pathway but it only functions in late S/G2 phase and then only rejoins a minor fraction of DSBs in G2. B-NHEJ represents a pathway that has been described to function in the absence of NHEJ. The thickness of the arrows above represent their importance for repairing DSBs generated by low doses of ionizing radiation.

Figure 1

Fig. 1

Pathways of double strand break repair



NHEJ.

- The most significant pathway repairing radiation-induced DSBs in mammalian cells
- Functions in G1, G2 and possibly S phase
- No requirement for homology.
- Fidelity unknown but no obvious ability to repair DSBs involving sequence loss and can potentially generate translocations

Defective mutants are dramatically radiation sensitive – little evidence that the proteins function in other processes

B-NHEJ

- Only functions in the absence of NHEJ proteins
- likely utilizes microhomology
- likely to involve sequence loss at the junctions

Defective mutants show little radiation sensitivity unless coupled with NHEJ deficiency.
Proteins function in SSB repair

HR

- Only functions in late S and G2 phase.
- In G2 repairs maximally 20 % of IR induced DSBs.
- Main function is to facilitate recovery from replication fork stalling
- Is able to repair DSBs with significant sequence loss at the junctions.
- Can generate translocation events

Defective mutants show mild radiation sensitivity

Proteins function at the replication fork to promote recovery from blocked replication

Figure 2



Features of the damage response mechanisms important for the maintenance of genomic stability in response to low doses/dose rates

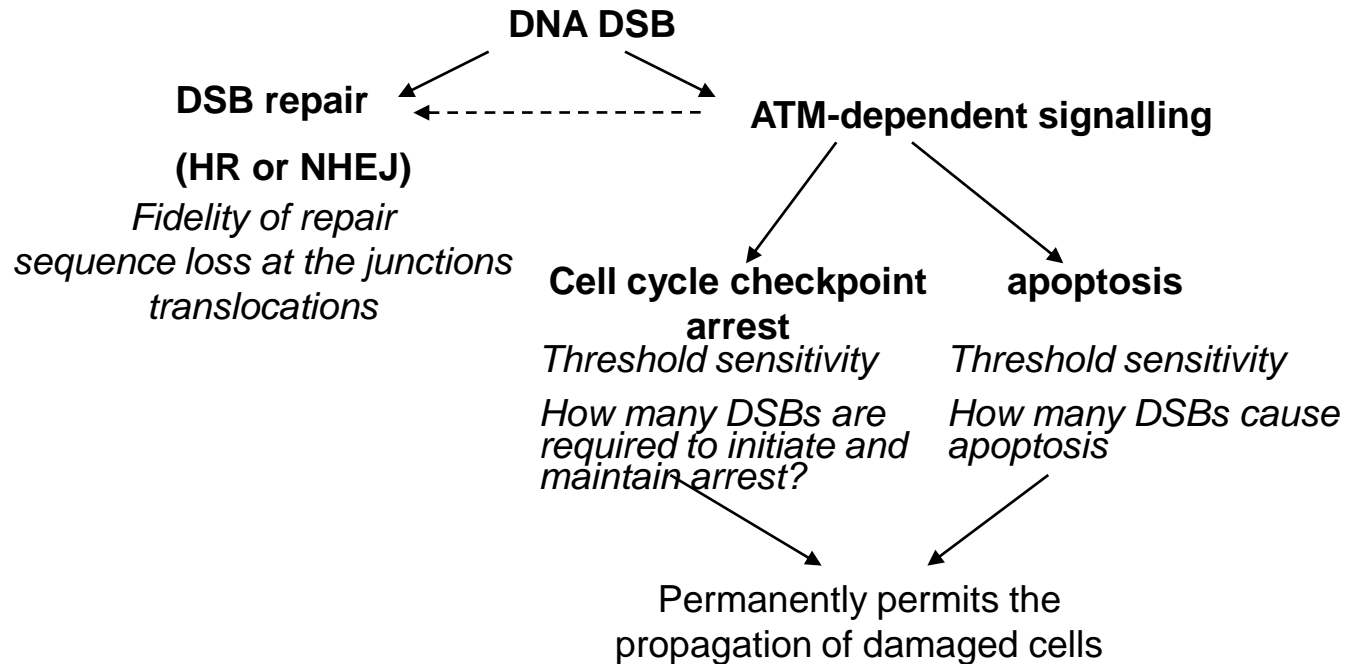
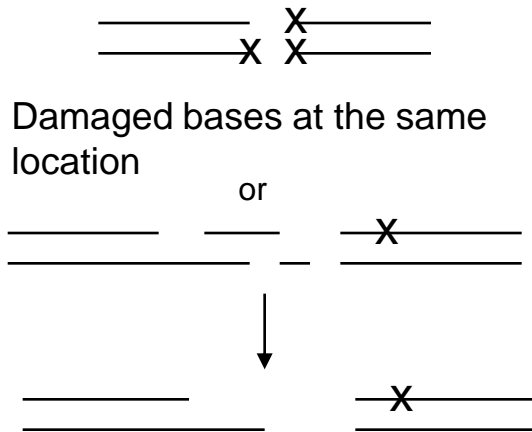


Fig. 2

Figure 3

Misrepair events that can be generated following exposure to IR.

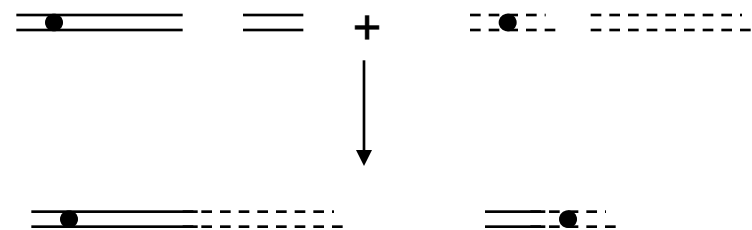
Loss of coding sequences



SSBs and DSBs in close proximity on one DNA molecule leading to fraying of the ends and loss of DNA sequence information

Generated by NHEJ but not HR

Generation of translocations



Translocation generated when DSBs arise in closely located chromosomes

Generated by NHEJ +HR

Fig. 3

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Date: 14/05/09
Subject: Transfer of Copyright Agreement, Submission to Health Physics
Message: Please find the requested above forms enclosed

Sincerely,

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