Mechanisms conferring sensitivity to low dose radiation exposure and consideration of potentially sensitive individuals.

Penny Jeggo

Genome Damage and Stability Centre, University of Sussex, Brighton, UK

BN1 9RQ.

Corresponding author: p.a.jeggo@sussex.ac.uk

Running title: potential radiosensitive individuals.

Abstract.

Here, I consider whether radiation sensitive individuals might exist in the population and the potential impact of low dose/dose rate radiation exposure. Radiation induces DNA double strand breaks (DSBs), which cause lethality if unrepaired and enhance genomic instability if misrepaired. DNA damage response (DDR) mechanisms play a vital role in protecting cells from the harmful effects of DSB formation. The DDR response encompasses DSB repair pathways, of which DNA non homologous end-joining is the most significant, and a signal transduction process involving ATM. Patients defective in DDR proteins have been described and some have shown clinical radiosensitivity. However, such patients are rare and belong to defined syndromes. The critical question is whether heterozygosity or mild defects in DDR proteins confer low dose radiosensitivity. Whilst it is unlikely that low dose radiation will dramatic enhance cell killing in individuals such patients, it is possible that there could be an impact on stem cell turnover leading to stem cell depletion with age. More importantly, it is likely that such patients could have increased misrepair of radiation damage and hence an elevated risk of radiation induced carcinogenesis. Evidence in support of this and the potentially important genes in this context are discussed.

The potentially harmful impact of low dose radiation damage.

Radiation induces a range of cellular damage, of which DNA damage is the most significant. Of the DNA lesions induced by radiation, a DNA double strand break (DSB), although not the most abundant lesion, is the most biologically significant. DSBs can be directly induced by radiation but can also arise indirectly following repair or replication of other lesions. Thus, here my focus will lie on the cellular mechanisms that respond to DSBs and the potential consequence of low level DSB formation. Following exposure to high doses of radiation, such as following accidental exposure or radiotherapy, individuals can show early acute hypersensitivity resulting from the inability of tissue stem cells to proliferate and maintain the physiological function of the organs, or more delayed hypersensitivity, which can potentially arise from tissue damage arising from an elevated inflammatory or cytokine signaling response. Clearly, impaired DSB response mechanisms represent one contributing process to both acute and delayed hypersensitivity. Hypersensitivity to low dose radiation exposure is less well understood but could potentially arise from impaired DNA damage responses or tissue damage. Here, I will focus on the potential hypersensitivity of individuals with impaired DNA damage response (DDR) mechanisms to low dose radiation exposure. In response to high radiation doses, the impact of radiation induced cell death is the most significant outcome for consideration. Whilst chronic low dose exposure could potentially also result in cell death and/or cellular senescence, another significant outcome for consideration is the onset of carcinogenesis. The potential hypersensitivity of individuals to carcinogenesis following low dose exposure represents an important component of the discussion below.

DNA damage response mechanisms of significance to low dose radiation exposure.

Since DSBs represent the most biologically significant radiation induced lesion, I will focus on the DNA damage response (DDR) mechanisms responding to DSBs. Two conceptually distinct pathways contribute to the DSB-DDR; DSB repair pathways function to repair the damage whilst a DSB signaling pathway senses the DSB and activates a range of distinct responses, including cell cycle checkpoint arrest and/or apoptosis. Although largely independent, the repair and DSB signaling response overlap since the signaling pathway regulates at least some components of DSB repair. DNA non homologous end-joining (NHEJ) represents the major DSB repair mechanism with homologous recombination (HR) being an additional mechanism that functions in late S/G2 phase of the cell cycle (*1*).

The process of NHEJ and its component proteins are shown in Figure 1. HR plays a minor role in repair of direct radiation induced DSBs but does have an important role in repairing DSBs that arise following replication of radiation induced lesions. Ataxia telangiectasia mutated (ATM) is a protein kinase that lies at the heart of the damage response signal transduction process that responds to DSBs (see Figure 2) (*2*). ATR, a related protein kinase, regulates a similar process that responds to replication fork stalling or other situations where single stranded DNA regions are generated. Although there is overlap between ATM and ATR, ATM is the major kinase that is activated by DSBs and thus will be the major focus here. ATM is rapidly recruited to DSB lesions most likely via the Mre11-Rad50-Nbs1 (MRN) complex (*3*). ATM is recruited as a dimer but following activation, undergoes autophosphorylation leading to monomerisation and full activation (*4*). An early step in the signaling cascade is phosphorylation of a histone H2A variant, H2AX, generating γ-H2AX (phosphorylated H2AX). A complex but ordered recruitment of "mediator" proteins then ensues, whose hierarchical and ordered assembly is just beginning to become understood (Fig 2) (see (*5*) for recent findings of the assembly process) . Currently, this process culminates in the retention of 53BP1 at the site of the DSB, although further downstream steps or components may yet be uncovered. The precise function of these proteins in ATM signaling is still not well understood but they appear to help to retain ATM at the DSB site and to serve, at least to some extent, to allow the signaling process to function in the face of the higher order chromatin structure of the DNA. Perhaps surprisingly, ATM activation and ATM-dependent phosphorylation events are only mildly impaired in cells lacking the mediator proteins, leading to the suggestion that they have an amplification role in ATM signaling (*6*). However, ATM

and the mediator proteins are specifically required for the repair of a subset of DSBs representing those located within or close to heterochromatic regions (*7*). These DSBs are repaired with slow kinetics in control cells, consistent with the notion that they require more complex processing.

As mentioned above, ATM signaling results in activation of cell cycle checkpoint arrest and/or apoptosis as well as regulating a component of DSB repair (*8*). Cell cycle checkpoint activation is achieved in part by the activation via phosphorylation of the transducer kinase, Chk2, as well as by phosphorylating p53, a transcriptional regulator. Another transducer kinase, Chk1, can also be activated in an ATM-dependent manner following ATM-dependent resection at DSBs and subsequent activation of ATR (*9*). For a consideration of the impact of ATM signaling on survival and genomic stability, rather than focusing further on the interesting but complex signaling process, I will consider the end points of the process, namely cell cycle checkpoint arrest and apoptosis. Apoptosis represents an ordered suicidal process that results in the destruction of damaged cells. Apoptosis plays a major role in removing cells during certain developmental processes, such as during lymphocyte development, but appears also to be exploited by the damage response to remove damaged cells that might otherwise survive and lead to carcinogenesis. The significantly elevated carcinogenesis and resistance to DNA damaging agents of p53 defective mice or cells, respectively, attests to the importance of apoptosis in preventing the proliferation of damaged cells. However, not all cells undergo apoptosis. Cell cycle checkpoint arrest represents the activation of surveillance mechanisms at “checkpoints” in the cell cycle to prevent progression through the cell cycle until critical processes have been completed (*10*). The process monitors DNA

integrity for the presence of DSBs (via ATM) or single stranded regions of DNA (via ATR). Checkpoint arrest can occur at the G1/S and G2/M transition and during S phase. The process primarily aims to prevent either replication or mitosis in the presence of DNA damage. Cell cycle checkpoint arrest can be transient providing enhanced time for DSB repair or can be permanent, thereby functioning as an alternative to apoptosis to prevent the proliferation of damaged cells.

Functional significance of the DSB DDR pathways.

DSBs are a particularly harmful lesion because, if unrepaired, loss of genetic material and most likely cell death, will ensue following cell division or, of equal significance, if misrepaired, DSBs can lead to genomic rearrangements with the potential activation of tumour activating genes or the loss of tumour suppressor genes, both events contributing to carcinogenesis. It is, therefore, important to consider not only how the DDR pathways enhance cellular survival but additionally how they serve to limit genomic instability. Indeed, for a consideration of the impact of low dose exposure, how the DDR pathways maintain genomic stability may be more important than how they enhance survival. The important role of NHEJ in enhancing cellular survival is demonstrated by the dramatic radiation sensitivity of mutants that lack NHEJ proteins (*11*). Although NHEJ is often described as being an inaccurate process, NHEJ mutants display elevated radiation induced chromosome rearrangements as well as increased chromosome breaks indicating that, even though errors may arise during NHEJ, the process is efficient in limiting chromosome rearrangements. Cell cycle checkpoint arrest and apoptosis both play

important roles in preventing the proliferation of damaged cells. Cell cycle checkpoint arrest also serves to increase the time for DSB repair, which can be particularly important for DSBs that are repaired with slow kinetics, a feature of DSBs induced by high linear energy transfer (LET) radiation. Failure to activate G2/M arrest after exposure to low doses of IR has been proposed to explain the phenomenon of low dose hypersensitivity, in which cells display relatively high sensitivity after exposure to low doses (less than 0.3 Gy) compared to the predicted response based on survival to a slightly higher dose (*12*). Whilst this possibility provides evidence that cell cycle checkpoint arrest can enhance survival, the process likely exerts its major impact on maintaining genomic stability since progression of cells through mitosis or replication decreases the possibility of accurate repair, and enhances the potential for misrepair. Importantly, however, the available data suggests that checkpoint arrest and DSB repair mechanisms function co-operatively to minimize genomic instability (*12*). This is most dramatically demonstrated in cell lines that lack ATM signalling, a process that impacts upon both DSB repair, cell cycle checkpoint arrest and apoptosis (*13*). Thus, ATM-defective cell lines display both high radiosensitivity and genomic instability.

Insight gained from the analysis of DDR disorders.

Significant defects in DSB DDR mechanisms have been described in patients conferring syndromes classified as DNA damage response disorders (see Table 1). Although such patients likely represent a subset of individuals with radiation hypersensitivity that manifests at low and high doses, the disorders are rare and normally associated with

marked clinical characteristics, which frequently result in shortened life expectancy. Nevertheless, a consideration of the clinical features of these patients and the phenotype of cell lines derived from them is informative in considering whether more subtle, but more common, defects in the DDR pathways might confer genetic susceptibility to low dose radiation.

Ataxia telangiestasia (A-T) is the human disorder conferred by loss of ATM, with many patients having completely inactivating (ie null) mutations (*8*). A-T is a devastating disorder conferring progressive neurodegeneration leading to ataxia, a variable level of immunodeficiency and cancer predisposition. Nijmegen Breakage Syndrome (NBS), A-T like disorder (ATLD) and the recently described Riddle Syndrome are further disorders that impact upon ATM signaling with defects in Nbs1, Mre11 and RNF168, respectively (*14-16*). Interestingly, whilst NBS patients display growth and developmental delay but not progressive ataxia, ATLD patients manifest a less severe A-T phenotype. However, the MRN complex is essential (ie null mutations do not exist) and is also required for efficient ATR signaling. The different phenotypes of these patients likely reflects the nature of the hypomorphic mutations in the genes, which may impact upon some but not all of the functions of the MRN complex. Defects in four components of the NHEJ machinery have also been reported in patients, namely DNA ligase IV, XLF, Artemis and DNA-PKcs (*17, 18*). These patients are characterized by severe combined immunodeficiency (SCID) or milder combined immunodeficiency due to the role that NHEJ plays during the process of V(D)J recombination. Another striking feature of some of the NHEJ defective patients is microcephaly, growth delay and dysmorphic facial features.

Cell lines derived from any of these patients display marked radiosensitivity, both to acute higher dose exposure as well as, where studied, to low dose or dose rate exposure (*19, 20*). In some cases, patients have shown clinical radiosensitivity following radiotherapy (*21*). The question, therefore, arises whether such patients would show hypersensitivity to low doses of IR. It should be appreciated that the phenotype of these patients arises as a consequence of diminished repair or signaling of endogenously arising DSBs (or DSBs that arise from background irradiation in our environment). Therefore, a consideration of their phenotypes might provide clues to the potential impact of low dose radiation exposure, where low numbers of excess unrepaired DSBs may accumulate.

A feature in common to these patients is cancer predisposition, with the most significant predisposition being to tumours of lymphoid origin. Paradoxically, the SCID conferred by more severe NHEJ defects precludes the onset of lymphoid tumours due to the diminished or absent T and B cells. However, in cases where residual T and B cells remain, lymphoid tumours are observed (*17*). This was strikingly shown in one LigIV syndrome patient who harboured a mild, hypomorphic mutation in DNA ligase IV that was not sufficiently severe to cause observable immunodeficiency (*21*). The patient, however, developed T cell leukaemia at age 14. The predisposition to lymphoid tumours could arise from a failure to adequately repair or respond to DSBs induced during immune development (V(D)J recombination or class switch recombination). Since the translocations that arise in these tumours require that two unrepaired DSBs rejoin incorrectly, it is likely that low dose radiation exposure will enhance lymphoid malignancy by increasing the background level of unrepaired DSBs. Such a possibility is consistent with the fact that radiation exposure enhances lymphoid malignancies. Similar

predisposition to lymphoid malignancies is also observed in mouse models of these disorders. These findings provide strong, albeit indirect, evidence that these patients may show hypersensitivity to carcinogenesis following low dose radiation exposure.

A further feature that is observed in some of the syndromes discussed above is microcephaly (*17*). The observed microcephaly occurs during embryonic development and is not progressive post birth. The analysis of wild type and LigIV syndrome mice (a mouse strain that, like the human patients, harbours a hypomorphic mutation in DNA ligase IV) suggests that the stem cells of the embryonic brain are dramatically radiation sensitive (Liu, unpublished observations). This, together with additional evidence, suggests that the embryonic developing brain may be particularly sensitive to exposure to low doses of radiation.

In recent work, Dr. M. Harms-Ringdahl has observed that human fibroblasts undergo premature growth arrest or senescence following exposure to chronic low dose rate irradiation (5 or 15 mGy/h) (unpublished observations). In collaborative work with the Harms-Ringdahl laboratory, we have observed that cells from A-T, LigIV or Artemis patients undergo senescence or growth arrest prematurely following chronic low dose irradiation compared to control cell lines (data not shown). This was most strikingly observed in LigIV syndrome cell lines where exposure to 5 mGy/h conferred rapid inhibition of cellular growth. This raises the possibility that low dose radiation exposure may confer premature loss of proliferative potential *in vivo* as a result of DSB accumulation exceeding the capacity of the cellular repair machinery. The findings also suggest that the DSB DDR disorder patients show a heightened response. Consistent with this, studies examining the haematopoeitic stem cell (HSCs) numbers of LigIV syndrome

mice provided strong evidence of diminished stem cell numbers with age as well as a reduced capacity to proliferate in grafting experiments compared to control HSCs (*22*). The evidence suggested that this was due to elevated unrepaired DSBs in the LigIV syndrome mice. Reduced stem cell proliferation potential was also observed in wild type mice following irradiation and by elevated levels of reactive oxygen species in ATM defective mice (*23, 24*). Together, these findings suggest that both radiation exposure to control mice or endogenously generated DSBs in DDR defective mice can inhibit the proliferation of HSCs. Whether low dose radiation exposure will confer a more marked impact has not yet been examined. Collectively, these studies raise the possibility that elevated levels of unrepaired DSBs may additionally confer a diminished capacity of stem cells to proliferate, which could confer stem cell depletion with age and/or premature ageing.

Can minor defects or polymorphisms in DDR proteins confer low dose radiation hypersensitivity.

As discussed above, individuals with DDR disorders are rare. The question, therefore, arises whether less impacting mutational changes or polymorphisms in these same genes confers hypersensitivity to low dose radiation exposure. It is possible that minor defects in the damage response proteins could impair the repair or processing of DSBs, thereby enhancing cell death and stem cell turnover. Perhaps, more importantly even a modest impairment of DDR protein function could potentially enhance the genomic rearrangements that can lead to carcinogenesis. This could arise via mis-repair of a

radiation induced DSB or by increasing the possibility of an interaction between and endogenously generated DSB, such as a DSB generated during immune development, and a radiation induced DSB.

Although the DNA damage response disorders are rare, heterozygous carriers of the strongly impacting mutational changes are, at least in some populations, present at up to 1 % of the population. Studies with heterozygous mice and cell lines derived from them have provided increasing evidence that heterozygosity for damage response proteins can, at least when coupled with other genetic defects, confer a subtle phenotype including cancer predisposition. For example, heterozygosity for DNA ligase IV in a tumour prone mouse strain, ink4a/art-/-, results in an elevated frequency of soft-tissue sarcomas with clonal amplifications, deletions and translocations (*25*). Additionally, dermal fibroblasts established from Ku80 heterozygous mice had elevated chromosome breaks (*26*). In addition, epidemiological studies on NBS and A-T heterozygotes, which in the Slavic and Ashkenazi Jewish populations, respectively, are relatively frequent, have provided evidence for elevated cancer predisposition with the impact being compounded by the effect of cigarette smoking (*27-31*). Finally, cellular studies on A-T heterozygous cell lines have also provided evidence of a detectably impaired damage response, which in one case was detected as a hypersensitivity to low dose radiation (*19, 20, 32*). A further patient class that should be mentioned in this context are heterozygous carriers of Brca1 or Brca2 mutations. Brca1 plays a role in ATM signaling and both proteins are required for HR. For these two genes, heterozygosity confers a marked predisposition to breast and ovarian cancer. Collectively, these and similar experiments suggest that even a two

fold reduction in the levels of damage response proteins is sufficient to drive chromosomal rearrangements leading to carcinogenesis.

The next question to consider is whether modest mutational changes in these damage response proteins, including polymorphic changes, can exert a two fold impact on function, without causing any overt features indicative of a damage response disorder. Patient 180BR was clinically normal but developed leukaemia at age 14 and subsequently dramatically overresponded to radiotherapy (*21*). A homozygous mutational change was identified in DNA ligase IV (R278H) which reduced activity by approximately 10 fold. Thus, even a 10 fold reduction in DNA ligase IV appears sufficient to preclude the pronounced immunodeficiency observed in LIG4 syndrome patients, although it was most likely causally related to the onset of leukaemia in the patient. Significantly, another LIG4 syndrome patient, who displayed marked immunodeficiency and developmental delay, was observed to harbour the same homozygous mutational change (R278H) as well as two closely linked polymorphic changes in DNA ligase IV. Strikingly, the activity of *in vitro* expressed DNA ligase IV carrying the linked polymorphisms was approximately two fold reduced compared to wild type DNA ligase IV and the impact of the polymorphisms on R278H DNA ligase IV was at least additive providing an explanation for their differing clinical features (*33*). This provides an example of a polymorphic change having a two fold impact on protein function and its potential impact on other genetic changes. Thus, an individual homozygous for this polymorphism would have a similar magnitude of reduced LigIV activity compared to a heterozygous carrier with a fully inactivating mutation in one allele. A further aspect that should be considered in this context is that some mutational changes exert a dominant negative impact on

protein function. Thus, although a mutational change may only partially impair protein function, it may have the capacity to inhibit the activity of the wild type protein. This occurs frequently for p53 mutations observed in cancers, and may occur, for example, in the case of the common founder mutation observed in NBS patients. Such a dominant negative effect has also been reported in knock-in mice for a mutation in ATM observed in A-T patients (*34*, *35*).

In the examples given above the cancer predisposition of heterozygous carriers of mutations in damage response genes was observed to spontaneous cancers and we cannot assess whether there may have been a contribution from exposure to background radiation exposure. In considering the likely additive or compounding impact of low dose radiation exposure, it is important to consider the distinction between DSBs induced by the byproducts of endogenous metabolism versus radiation induced DSBs. Endogenously introduced DSBs can arise from reactive oxygen species (ROS) as well as during defined metabolic processes such as V(D)J recombination. Abundant data has shown that radiation damage can be more complex than such endogenously arising DSBs and as a consequence have an increased potential for misrepair. In this context, any level of radiation damage, and most importantly, any exposure to high LET radiation, could be considered to be distinct from endogenous damage. Given the significant role that the DDR proteins discussed above play in the response to radiation exposure, it is reasonable to argue that the impact for cancer induction caused by low dose radiation exposure will be at least additive to that arising from endogenously arising DSBs. The studies of Kato et al. showing elevated levels of unrepaired DSBs in fibroblasts derived from A-T

heterozygotes following low dose rate exposure is particularly pertinent in this context (*19, 20*).

Conclusions.

Here, I have overviewed the significant role that the DDR proteins that respond to DSBs play in enhancing survival and maintaining genomic stability in response to DSB formation. The most significant processes in this context are those that function in DNA non-homologous end-joining, homologous recombination and ATM signaling. Low dose radiation exposure may not dramatically impact upon cell killing although it is possible that chronic low dose rate exposure could lead to depletion of certain stem cell compartments with age. However, a highly significant role that the DDR proteins play in response to low dose radiation is to maintain genomic stability, thereby limiting the potential for the chromosome rearrangements that can drive carcinogenesis. Thus, one class of individuals who are likely to display genetic predisposition to the harmful effects of low dose exposure will be those with severe mutations in the DSB-DDR proteins, as observed in the characterized DNA damage response disorders. Fortunately, such individuals are rare. However, there is mounting evidence from mice and human studies that even a two fold reduction in the levels of such proteins as observed in heterozygous carriers can confer significant cancer predisposition. Further, I provide at least one example where polymorphic changes can provide a two fold impact on protein function. Given the known role of these proteins in maintaining genomic stability following radiation exposure, it is likely that individuals harbouring more modestly impacting

mutations in the DSB-DDR genes have the potential to display a predisposition to cancer induction from low dose radiation exposure and may thus represent a genetically sensitive sub-population.

References.

1. C. Wyman and R. Kanaar, DNA double-strand break repair: all's well that ends well. *Annu Rev Genet* **40**, 363-383 (2006).

2. E. U. Kurz and S. P. Lees-Miller, DNA damage-induced activation of ATM and ATM-dependent signaling pathways. *DNA Repair (Amst)* **3**, 889-900 (2004).

3. T. Uziel, Y. Lerenthal, L. Moyal, Y. Andegeko, L. Mittelman and Y. Shiloh, Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J.* **22**, 5612-5621 (2003).

4. C. J. Bakkenist and M. B. Kastan, DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* **421**, 499-506 (2003).

5. C. Doil, N. Mailand, S. Bekker-Jensen, P. Menard, D. H. Larsen, R. Pepperkok, J. Ellenberg, S. Panier, D. Durocher, et al., RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. *Cell* **136**, 435-446 (2009).

6. O. Fernandez-Capetillo, H. T. Chen, A. Celeste, I. Ward, P. J. Romanienko, J. C. Morales, K. Naka, Z. Xia, R. D. Camerini-Otero, et al., DNA damage-induced G2-M checkpoint activation by histone H2AX and 53BP1. *Nat Cell Biol* **4**, 993-997. (2002).

7. A. A. Goodarzi, A. T. Noon, D. Deckbar, Y. Ziv, Y. Shiloh, M. Lobrich and P. A. Jeggo, ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. *Mol Cell* **31**, 167-177 (2008).

8. M. F. Lavin, Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer. *Nat Rev Mol Cell Biol* **9**, 759-769 (2008).

9. A. Jazayeri, J. Falck, C. Lukas, J. Bartek, G. C. Smith, J. Lukas and S. P. Jackson, ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. *Nat Cell Biol* **8**, 37-45 (2006).

10. T. Caspari and A. M. Carr, Checkpoints: how to flag up double-strand breaks. *Curr Biol.* **12**, R105-107 (2002).

11. P. A. Jeggo and M. Lobrich, Contribution of DNA repair and cell cycle checkpoint arrest to the maintenance of genomic stability. *DNA Repair (Amst)* **5**, 1192-1198 (2006).

12. M. Lobrich and P. A. Jeggo, The impact of a negligent G2/M checkpoint on genomic instability and cancer induction. *Nat Rev Cancer* **7**, 861-869 (2007).

13. M. Lobrich and P. A. Jeggo, Harmonising the response to DSBs: a new string in the ATM bow. *DNA Repair (Amst)* **4**, 749-759 (2005).

14. R. Varon, C. Vissinga, M. Platzer, K. M. Cerosaletti, K. H. Chrzanowska, K. Saar, G. Beckmann, E. Seemanova, P. R. Cooper, et al., Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. *Cell* **93**, 467-476 (1998).

15. G. S. Stewart, R. S. Maser, T. Stankovic, D. A. Bressan, M. I. Kaplan, N. G. Jaspers, A. Raams, P. J. Byrd, J. H. Petrini and A. M. Taylor, The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell* **99**, 577-587 (1999).

16. G. S. Stewart, S. Panier, K. Townsend, A. K. Al-Hakim, N. K. Kolas, E. S. Miller, S. Nakada, J. Ylanko, S. Olivarius, et al., The RIDDLE syndrome protein

mediates a ubiquitin-dependent signaling cascade at sites of DNA damage. *Cell* **136**, 420-434 (2009).

17. M. O'Driscoll and P. A. Jeggo, The role of double-strand break repair - insights from human genetics. *Nat Rev Genet* **7**, 45-54 (2006).

18. M. van der Burg, H. Ijspeert, N. S. Verkaik, T. Turul, W. W. Wiegant, K. Morotomi-Yano, P. O. Mari, I. Tezcan, D. J. Chen, et al., A DNA-PKcs mutation in a radiosensitive T-B- SCID patient inhibits Artemis activation and nonhomologous end-joining. *J Clin Invest* **119**, 91-98 (2009).

19. T. A. Kato, H. Nagasawa, M. M. Weil, J. B. Little and J. S. Bedford, Levels of gamma-H2AX Foci after low-dose-rate irradiation reveal a DNA DSB rejoining defect in cells from human ATM heterozygotes in two at families and in another apparently normal individual. *Radiat Res* **166**, 443-453 (2006).

20. T. A. Kato, H. Nagasawa, M. M. Weil, P. C. Genik, J. B. Little and J. S. Bedford, gamma-H2AX foci after low-dose-rate irradiation reveal atm haploinsufficiency in mice. *Radiat Res* **166**, 47-54 (2006).

21. E. Riballo, S. E. Critchlow, S. H. Teo, A. J. Doherty, A. Priestley, B. Broughton, B. Kysela, H. Beamish, N. Plowman, et al., Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. *Curr Biol* **19**, 699-702 (1999).

22. A. Nijnik, L. Woodbine, C. Marchetti, S. Dawson, T. Lambe, C. Liu, N. P. Rodrigues, T. L. Crockford, E. Cabuy, et al., DNA repair is limiting for haematopoietic stem cells during ageing. *Nature* **447**, 686-690 (2007).

23. Y. Wang, B. A. Schulte, A. C. LaRue, M. Ogawa and D. Zhou, Total body irradiation selectively induces murine hematopoietic stem cell senescence. *Blood* **107**, 358-366 (2006).

24. K. Ito, A. Hirao, F. Arai, S. Matsuoka, K. Takubo, I. Hamaguchi, K. Nomiyama, K. Hosokawa, K. Sakurada, et al., Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. *Nature* **431**, 997-1002 (2004).

25. N. E. Sharpless, D. O. Ferguson, R. C. O'Hagan, D. H. Castrillon, C. Lee, P. A. Farazi, S. Alson, J. Fleming, C. C. Morton, et al., Impaired nonhomologous end-joining provokes soft tissue sarcomas harboring chromosomal translocations, amplifications, and deletions. *Mol Cell* **8**, 1187-1196 (2001).

26. Z. E. Karanjawala, U. Grawunder, C. L. Hsieh and M. R. Lieber, The nonhomologous DNA end joining pathway is important for chromosome stability in primary fibroblasts. *Curr Biol* **9**, 1501-1504 (1999).

27. M. Swift and J. L. Lukin, Breast cancer incidence and the effect of cigarette smoking in heterozygous carriers of mutations in the ataxia-telangiectasia gene. *Cancer Epidemiol Biomarkers Prev* **17**, 3188-3192 (2008).

28. D. Thompson, S. Duedal, J. Kirner, L. McGuffog, J. Last, A. Reiman, P. Byrd, M. Taylor and D. F. Easton, Cancer risks and mortality in heterozygous ATM mutation carriers. *J Natl Cancer Inst* **97**, 813-822 (2005).

29. J. Nowak, M. Mosor, I. Ziolkowska, M. Wierzbicka, M. Pernak-Schwarz, M. Przyborska, K. Roznowski, A. Plawski, R. Slomski and D. Januszkiewicz, Heterozygous carriers of the I171V mutation of the NBS1 gene have a significantly increased risk of solid malignant tumours. *Eur J Cancer* **44**, 627-630 (2008).

30. E. Seemanova, P. Jarolim, P. Seeman, R. Varon, M. Digweed, M. Swift and K. Sperling, Cancer risk of heterozygotes with the NBN founder mutation. *J Natl Cancer Inst* **99**, 1875-1880 (2007).

31. N. Bogdanova, S. Feshchenko, P. Schurmann, R. Waltes, B. Wieland, P. Hillemanns, Y. I. Rogov, O. Dammann, M. Bremer, et al., Nijmegen Breakage Syndrome mutations and risk of breast cancer. *Int J Cancer* **122**, 802-806 (2008).

32. S. A. Nahas, A. W. Butch, L. Du and R. A. Gatti, Rapid flow cytometry-based structural maintenance of chromosomes 1 (SMC1) phosphorylation assay for identification of ataxia-telangiectasia homozygotes and heterozygotes. *Clin Chem* **55**, 463-472 (2009).

33. P.-M. Girard, B. Kysela, C. J. Harer, A. J. Doherty and P. A. Jeggo, Analysis of DNA ligase IV mutations found in LIG4 syndrome patients: the impact of two linked polymorphisms. *Human Molecular Genetics* **13**, 2369-2376 (2004).

34. M. Mitui, S. A. Nahas, L. T. Du, Z. Yang, C. H. Lai, K. Nakamura, S. Arroyo, S. Scott, A. Purayidom, et al., Functional and computational assessment of missense variants in the ataxia-telangiectasia mutated (ATM) gene: mutations with increased cancer risk. *Hum Mutat* **30**, 12-21 (2009).

35. K. Spring, F. Ahangari, S. P. Scott, P. Waring, D. M. Purdie, P. C. Chen, K. Hourigan, J. Ramsay, P. J. McKinnon, et al., Mice heterozygous for mutation in Atm, the gene involved in ataxia-telangiectasia, have heightened susceptibility to cancer. *Nat Genet* **32**, 185-190 (2002).

Figure Legends:

Figure 1: The process of DNA non homologous end-joining.

The first step of DNA NHEJ (1) involves the binding of the heterodimeric Ku protein to DNA ends. Many of the DNA ends caused by radiation damage may have associated base damage as shown by the triangle and star on the DNA ends. Ku is a basket shaped molecule and encircles the DNA. It also has the capacity to translocate along the DNA. The second step of the reaction (2) involves the recruitment of DNA-PKcs to Ku-bound DNA generating the DNA-PK complex. DNA-PKcs is a larger protein with a kinase domain at its C-terminus. Binding of DNA-PKcs to DNA-bound Ku activates its kinase activity. The role of the DNA-PK kinase activity is currently unclear but the protein undergoes autophosphorylation. There is mounting evidence that DNA-PK facilitates processing of the DNA ends. Finally, a ligation complex involving a complex of DNA ligase IV and Xrcc4, and the associated protein, XLF, effects the ligation step of the reaction (3) to generate intact DNA (4).

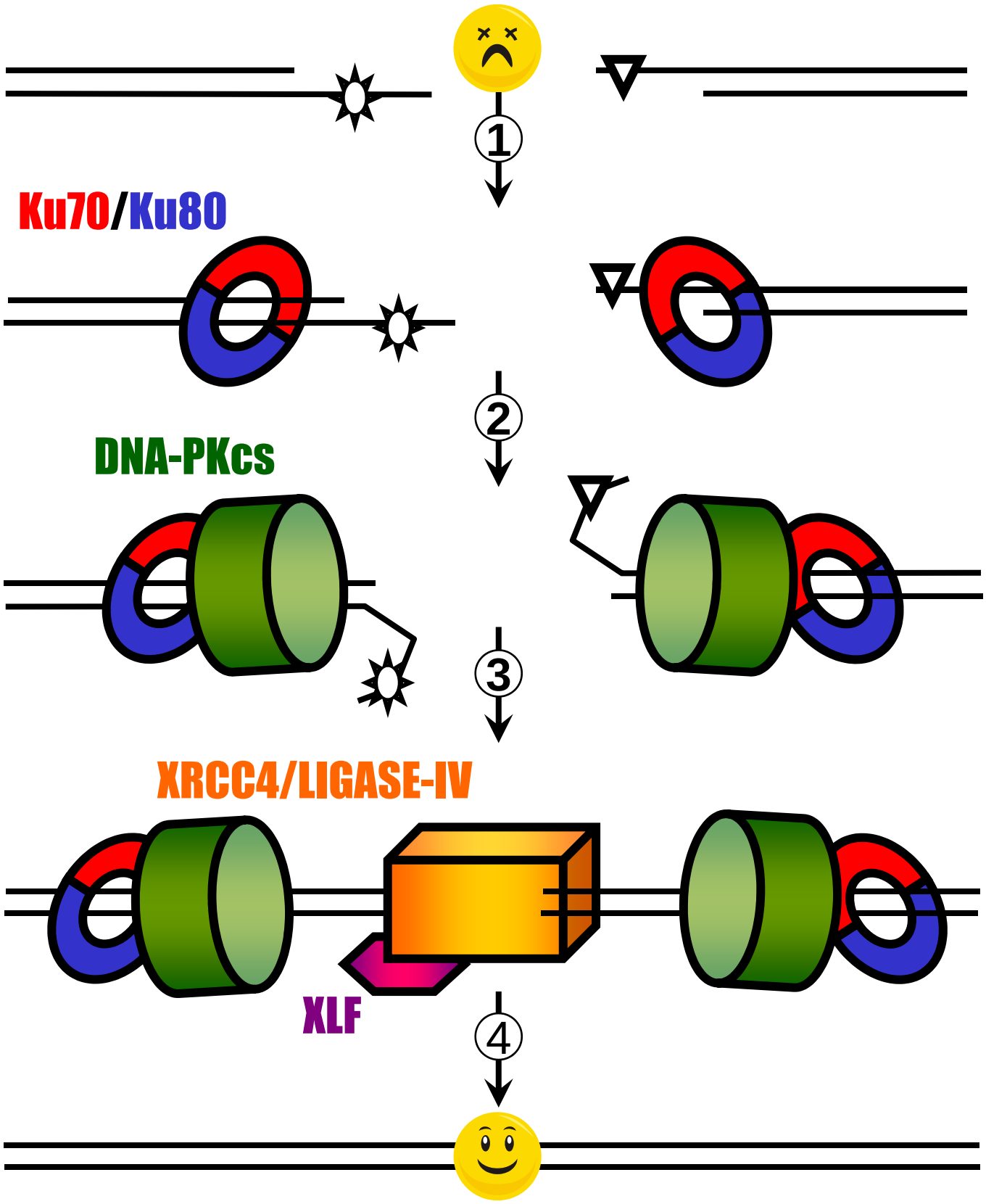
Figure 2: The process of ATM signaling and mediator protein recruitment.

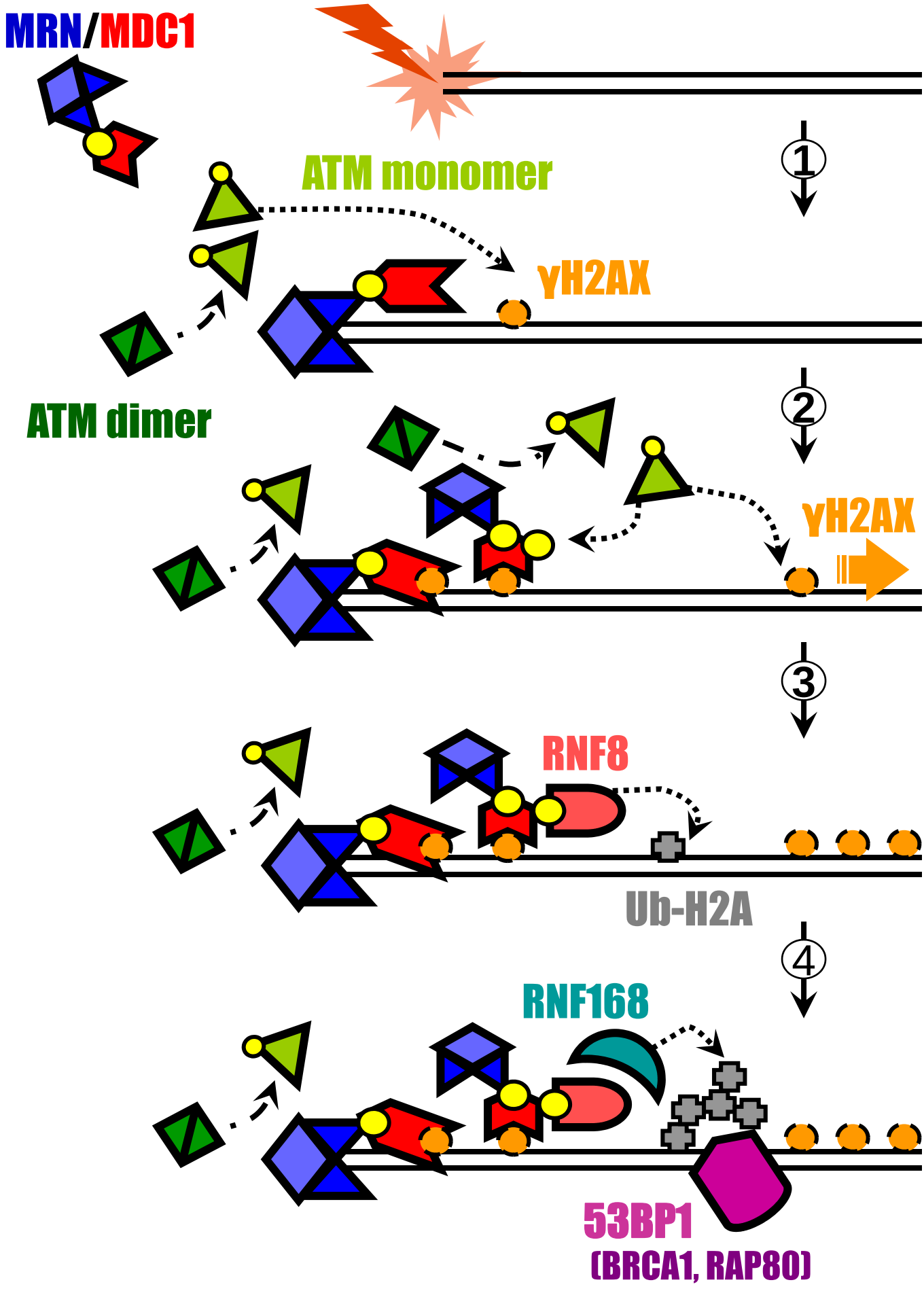
Current evidence suggests that the Mre11/Rad50/Nbs1 (MRN) complex (shown in blue) represents the primary DSB sensor which recruits dimerised ATM to the DNA end (step 1). This rapidly causes ATM monomerisation and activation. An early substrate of ATM is H2AX, a variant from of the histone, H2A. Phosphorylated H2AX is designated g-H2AX. MRN is also associated with MDC1 (shown in red). ATM phosphorylation of MDC1/H2AX enhances the binding of MDC1.to H2AX allowing it to be stably

maintained at the DNA end (step 2). This results in the recruitment of the ubiquitin ligase, RNF8 (step 3) and subsequently RNAF168, (step 4) which ubiquitinate H2A. This in turn promotes the recruitment and retention of 53BP1 (shown in pink) at the DSB end. Recent evidence has also suggested that Brca1 and Rap80 contribute to H2A ubiquitination, and help with 53BP1 retention at the DNA end. Retention of 53BP1 appears to be dispensable for ATM activation but helps to retain ATM at the DSB site to promote localized phosphorylation events (Noon, Goodarzi and Jeggo, unpublished observations).

Table 1.
Overview of clinical disorders associated with defects in DNA double strand break repair and/or signaling.

Syndrome	Defective Gene	Radiation sensitivity	Clinical features	Roles
Ataxia telangiectasia (A-T)	ATM	+++	Mild immunodeficiency Progressive ataxia Cancer predisposition	ATM signaling. Subfraction of DSB repair
Nijmegen Breakage Syndrome (NBS)	Nbs1	++	Mild immunodeficiency Microcephaly/ growth delay	ATM signaling + essential role. Subfraction of DSB repair
Ataxia telangiectasia like disorder (ATLD)	Mre11	++	Mild A-T like features	as for Nbs1
Riddle Syndrome	RNF168	++	Mild immunodeficiency Growth and developmental delay	ATM signaling Subfraction of DSB repair
LigIV Syndrome	DNA ligase IV	++	SCID or CID Microcephaly and growth delay	NHEJ
XLF Syndrome	XLF/Cernunnos	++	SCID or CID Microcephaly /growth delay	NHEJ
DNA-PKcs deficiency	DNA-PKcs	++	CID	NHEJ
RS-SCID1	Artemis	++	SCID	Subfraction of DSB repair


Ku70/Ku80
DNA-PKcs
XRCC4/LIGASE-IV
XLF
1
2
3
4


MRN/MDC1
ATM monomer
①
γH2AX
ATM dimer
②
γH2AX
③
RNF8
Ub-H2A
④
RNF168
53BP1
(BRCA1, RAP80)