



The DNA damage response, genome instability and cancer

The research in our laboratory focuses on understanding the cellular DNA damage response (DDR) and its role in safeguarding human health. This response to diverse DNA lesions is crucial for maintaining the stability of the genome. Genome instability and a defective DDR are a hallmark of cancer cells and can predispose to cancers and other debilitating pathologies. Detection of DNA damage leads to changes in cell behaviour, including cell cycle checkpoint arrest and in some cases the activation of cell death pathways. We investigate the molecular signalling pathways that control these responses in order to understand their role in tumour suppression and to identify ways to manipulate them for clinical applications.

The DNA damage response in human disease

The integrity of the genome is threatened by diverse types of DNA damage. This damage can originate from cellular processes, such as DNA replication, or result from exposure to exogenous environmental insults, such as ionizing radiation or chemical mutagens. If not dealt with appropriately, unrepaired DNA damage can be amplified or exacerbated during cell division, cause defects in cellular function, and result in the generation of potentially oncogenic mutations or rearrangements.

The detection of DNA lesions by damage-sensing proteins activates the DNA damage response (DDR), triggering signal trans-

duction cascades that alter cell behaviour to promote genome stability (Figure 1). These alterations include the activation of cell cycle checkpoints, the recruitment and/or activation of appropriate repair factors, and, in some cases, the initiation of specialised responses such as programmed cell death (apoptosis) or permanent exit from the cell cycle (senescence). These mechanisms prevent DNA damage from being passed on to daughter cells and are known to play crucial roles in tumour suppression.

Different types of DNA lesions require different repair pathways and affect cell behaviour in distinct ways. DNA double strand breaks (DSBs) are considered one of the most dangerous types of

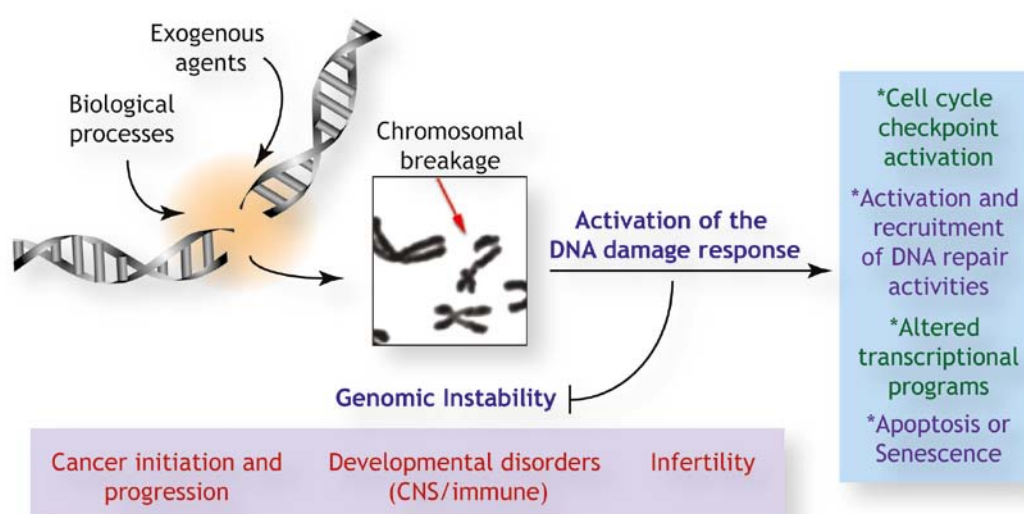
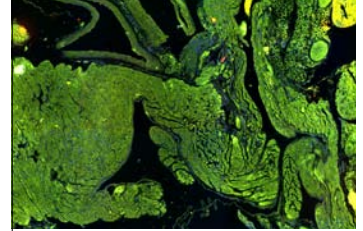


Figure 1. The activation of the DNA damage response. Chromosomal breakage arising from endogenous or exogenous sources activates the DNA damage response. This leads to changes in cell behaviour (blue box) that prevent the accumulation of genomic instability that can promote various human pathologies (violet box), including cancer.



DNA damage because they can be cytotoxic or cytostatic and can lead to the generation of oncogenic translocations if not repaired correctly. Defects in the DDR to DSBs underlies many severe human hereditary disorders that predispose patients to cancer and other serious pathologies. For example, the human disorders Ataxia-telangiectasia (A-T) and Nijmegen Breakage Syndrome (NBS) are characterised by DSB sensitivity, predisposition to cancer, immunodeficiency, and severe neuronal pathology. In addition to hereditary cancer predisposition, genes encoding proteins that respond to DSBs are often found mutated in spontaneous human cancers.

DNA DSBs and the G2/M cell cycle checkpoint

DSBs are rapidly detected by the highly conserved Mre11-Rad50-Nbs1 (MRN) sensor complex. Mutations in the genes that encode all 3 members of the complex have been found to underlie human genetic instability syndromes (reviewed by Lavin, 2007). Recognition of DSBs by MRN activates signal transduction kinases, such as Ataxia-telangiectasia mutated (ATM). Both the MRN complex and ATM play important roles in the regulation of cell cycle checkpoints, particularly during S-phase DNA replication and in G2.

Previously, we have employed genetic approaches to identify the role of the MRN complex in tumour suppression (Stracker *et al*, 2007; Stracker *et al*, 2008). These studies implicated the G2/M cell cycle checkpoint activities of MRN as playing a crucial role. This checkpoint is activated following DNA damage and prevents G2 cells from initiating mitosis with broken chromosomes. This checkpoint has also received considerable interest in clinical research as the ability to inactivate it during chemotherapy can enhance the toxicity of DNA-damaging drugs on dividing tumour cells.

Activation of the G2/M checkpoint following DSB detection requires the processing of double-stranded DNA (dsDNA) breaks 5' to 3' to form single-stranded DNA (ssDNA) tails at the break sites, a process called resection. The formation of ssDNA tails is a crucial event for both cell cycle checkpoint activation as well as homologous recombination-mediated repair of DNA breaks. Resection of dsDNA to ssDNA requires

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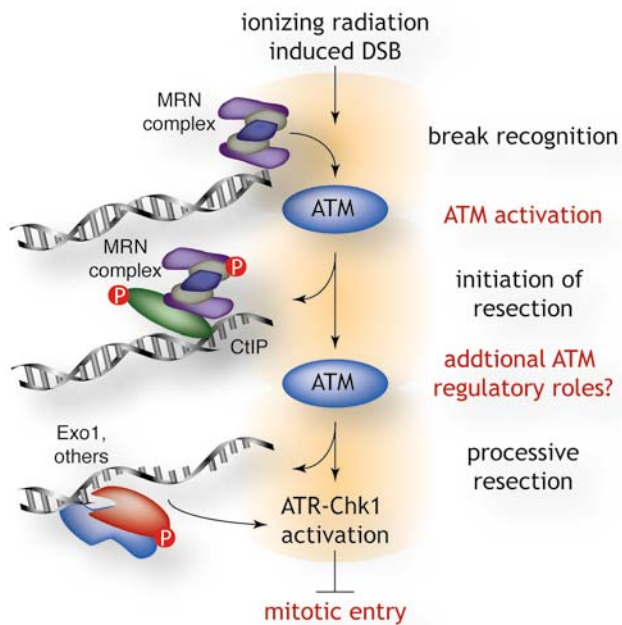


Figure 2. The activation of the G2/M checkpoint. Detection of breaks by the MRN complex leads to the activation of ATM kinase activity. The MRN complex and CtIP are substrates of ATM and initiate resection at DSB ends. Following short resection of the ends, additional factors such as Exo1 continue the resection process. Many of these proteins are substrates of ATM, thereby suggesting a regulatory role. Resection is required for the efficient activation of the ATR and Chk1 kinases that inhibit mitotic entry through modulation of cyclin-dependent kinase activity.

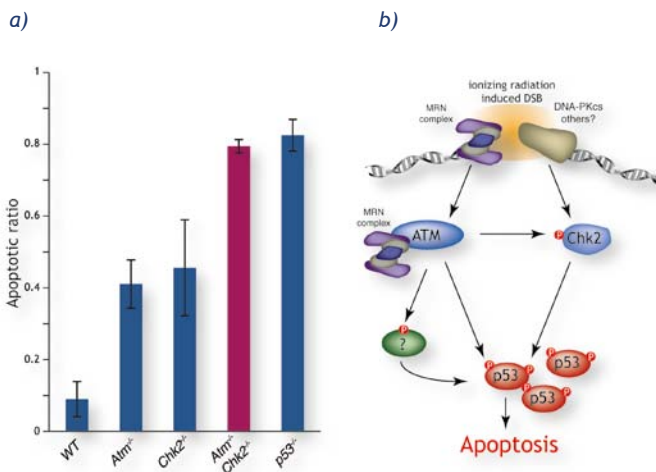


Figure 3. Apoptotic signalling in thymocytes. (a) Analysis of apoptosis in primary thymocytes 20 hours following exposure to 5 Gy of ionizing radiation. Genetic analysis revealed that Chk2 is required for ATM independent apoptosis in response to ionizing radiation (from Stracker et al, 2007). (b) Working model of apoptosis in thymocytes. The MRN complex activates ATM and plays a secondary role in the phosphorylation of some ATM substrates required for apoptosis. Chk2 is phosphorylated by ATM but additional kinases can activate Chk2 in the absence of ATM. DNA-PKcs is a prime candidate for this activity and promotes ATM independent apoptosis (Callén et al, 2009).

the concerted action of multiple nucleases, helicases and regulatory proteins. It remains unclear as to precisely which enzymatic activities are required for resection but recent advances in yeast and biochemical systems have provided much insight and resulted in a 2-step model of resection, summarized in Figure 2 (Mimitou and Symington, 2009). The MRN complex binds DSBs and recruits Sae2 (CtIP in mammalian cells) to promote the first step, removing a short stretch of nucleotides from the break ends. This is then followed by processive resection, which can be carried out by the Exo1 nuclease and a helicase or the DNA2 protein that has both nuclease and helicase activities. Biochemical data in mammalian cells implicate many of the same activities in the resection process but their roles in checkpoint activation remain unclear.

The observation that the activation of the ATM kinase is crucial for checkpoint activation in G2 suggests that ATM participates in regulating resection. Consistent with this, many of the enzymatic activities involved, including the MRN complex members, CtIP and Exo1, are substrates of ATM and related kinases. In ATM-deficient cells, the activation of the G2/M checkpoint is severely impaired and this correlates with reduced activity of the ATR and Chk1 kinases known to be required for G2 arrest. In cells expressing hypomorphic Nbs1 mutants that lack the N-terminal FHA and BRCT phospho-peptide binding domains, the G2/M checkpoint is impaired, but to a lesser degree than in ATM mutants. These Nbs1 mutants are expressed at very low levels and structural studies have shown that the N-terminal domains of Nbs1 are important for interactions with CtIP, another protein that is also crucial for initiating resection (Williams et al, 2009). Thus, the mild G2/M defect in Nbs1 mutant cells is surprising. In yeast, deletion of both Nbs1 and Exo1 leads to a synthetic growth defect and mutation of both genes produces more severe resection defects in the resection process (Mimitou and Symington, 2009). A potential explanation for the less severe G2/M checkpoint defect of Nbs1 mutants compared to ATM null mammalian cells is that partially redundant activities, such as Exo1, suppress the defects of Nbs1 mutants. Consistent with this possibility, Exo1 has been identified as a substrate of ATM/ATR activity and its overexpression can enhance ATR-Chk1 activation in response to synthetic oligonucleotide substrates (Shiotani and Zou, 2009).

In 2009 we have initiated projects to examine the mechanism of DNA resection and G2/M checkpoint activation. We are testing the hypothesis that Exo1 suppresses the severity of checkpoint defects in Nbs1 mutants. We are doing this using multiple cell culture-based approaches to determine the relevance of Exo1 in normal and Nbs1 mutant backgrounds during G2/M checkpoint induction. Complementary genetic and biochemical approaches have also been initiated to identify proteins involved in the resection process that may provide insight into its regulation following DSB detection.

The activation of radiation-induced apoptosis

Exposure to ionizing radiation results in severe toxicity characterized by cell death in both the hematopoietic system and the gastrointestinal tract. While occupational exposure to high doses of radiation is rare, ionizing radiation is frequently used as

a therapy for cancer. The Chk2 kinase that is activated following DSB recognition is important for preventing radiation toxicity (reviewed in Stracker *et al*, 2009). We, and others, have shown it is a tumour suppressor in the context of particular mutations that affect genome stability (Stracker *et al*, 2008). While Chk2 activation is well understood at the biochemical level, we have identified alternative pathways of Chk2 activation that are important for radiation-induced apoptosis and may play a role in tumour suppression.

Following DSB recognition by MRN, the Chk2 kinase is rapidly phosphorylated in trans by the ATM kinase that promotes its homodimerization and trans autophosphorylation. This MRN-ATM dependent activation of Chk2 is believed to be the primary mechanism of Chk2 activation following radiation exposure. In previous studies we found unexpectedly that Chk2 dependent apoptosis was still active in cells lacking ATM or in Mre11 hypomorphic mutants that fail to activate ATM properly (Stracker *et al*, 2007; Stracker *et al*, 2008). We have found that damage induced Chk2 phosphorylation occurs rapidly after ionizing radiation treatment in the absence of ATM, implicating additional kinases in the apoptotic response. We are currently investigat-

ing the kinase(s) responsible for ATM-independent activation of Chk2 in order to better understand how apoptosis is initiated after radiation exposure. DNA-PKcs is a prime candidate for alternative activation of Chk2 in apoptosis as it has been shown to be required for apoptosis in ATM deficient cells and modifies Chk2 *in vitro*. Our current working model is presented in Figure 3.

Concluding remarks

Our laboratory is taking a wide array of approaches to better understand aspects of the cellular response to DNA damage that have been linked to cancer prevention. Available data implicates both the G2/M checkpoint and apoptosis as playing central roles in tumor suppression. Understanding the molecular events that take place during these processes and how they influence cell behavior will potentially identify new prognostic markers for cancer screening and identification. Additionally, both checkpoint arrest and apoptosis influence the efficacy and toxicity of many chemotherapeutic approaches. Thus the identification of novel factors and regulatory mechanisms that influence these processes may open new inroads to enhance the potency and safety of current cancer treatment regimens.

Scientific output

Publications

Stracker TH, Usui T and Petrini JH. Taking the time to make important decisions: the checkpoint effector kinases Chk1 and Chk2 and the DNA damage response. *DNA Repair*, **8**(9), 1047-54 (2009)

Other references

Callén E, Jankovic M, Wong N, Zha S, Chen HT, Difilippantonio S, Di Virgilio M, Heidkamp G, Alt FW, Nussenzweig A and Nussenzweig M. Essential role for DNA-PKcs in DNA double-strand break repair and apoptosis in ATM-deficient lymphocytes. *Mol Cell*, **34**(3), 285-97 (2009)

Lavin MF. ATM and the Mre11 complex combine to recognize and signal DNA double-strand breaks. *Oncogene*, **26**(56), 7749-58 (2007)

Mimitou EP and Symington LS. DNA end resection: many nucleases make light work. *DNA Repair*, **8**(9), 983-95 (2009)

Shiotani B and Zou L. Single-stranded DNA orchestrates an ATM-to-ATR switch at DNA breaks. *Mol Cell*, **33**(5), 547-58 (2009)

Stracker TH, Morales M, Couto SS, Hussein H and Petrini JH. The carboxy terminus of NBS1 is required for induction of apoptosis by the MRE11 complex. *Nature*, **447**(7141), 218-21(2007)

Stracker TH, Couto SS, Cordon-Cardo C, Matos T and Petrini JH. CHK2 suppresses the oncogenic potential of DNA replication-associated DNA damage. *Mol Cell*, **31**(1), 21-32 (2008)

Williams RS, Dodson GE, Limbo O, Yamada Y, Williams JS, Guenther G, Classen S, Glover JN, Iwasaki H, Russell P and Tainer JA. Nbs1 flexibly tethers Ctp1 and Mre11-Rad50 to coordinate DNA double-strand break processing and repair. *Cell*, **139**(1), 87-99 (2009)

Research networks and grants

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Collaborations

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