A focus on foci: How spatio-temporal regulation of the DNA damage response is crucial for DDR activation and cell fate

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Summary

DNA damage is a continuous threat to cells during their lifetime and it can be resolved through activation of the DNA damage response (DDR). This activation is a complicated process which involves the accumulation of a variety of DDR factors. Specific factors assemble at the site of the lesion, which is a crucial step in DDR activation. Next, the signal is amplified and more DDR factors are recruited which leads to spreading of the signal into the surrounding chromatin and transduction throughout the nucleus. This review will focus on how this spatio-temporal organization of the DDR is crucial for activation and on recent work that has showed that artificially localizing DDR factors to the chromatin induces a DDR without the need of an actual DNA lesion. This approach presents the DDR as a dynamic signaling cascade which is not only required for initiating DNA repair but can also influence cell fate. Unresolved DNA damage causes persistent signaling which leads to cell cycle arrest and can ultimately induce senescence. Because the DDR has an important role in guarding malignant transformation, this knowledge could provide more insight into how the DDR can guide premalignant lesions into senescence and thereby prevent the development of cancer.

Introduction

DNA damage is a continuous threat to cells during their lifetime. Exogenous sources like ultra-violet (UV) radiation, toxic chemicals and cellular processes like DNA replication and metabolism are causes of DNA damage. Unrepaired DNA damage interferes with cellular processes like DNA replication and transcription and this potentially compromises the genomic integrity of the cell. Besides, this could result into chromosomal aberrations or mutations that may ultimately result into the development of cancer. To repair DNA damage, the cell has developed an intricate response network that allows for DNA repair by activating a signaling cascade that results into a transient cell cycle arrest. This DNA damage response (DDR) is very important for the viability of the cell and mutations in genes involved in this pathway could lead to various pathological disorders, for example neurodegenerative diseases, developmental defects and cancer predisposition (Ciccia and Elledge, 2010; Polo and Jackson, 2011a)

Among the different types of DNA damage are crosslinked bases, pyrimidine dimers and single or double-stranded breaks (Hoeijmakers, 2001). These lesions are either formed spontaneously after an error during DNA replication, or they are formed after exposure to sources like reactive oxygen species (ROS) (Cooke et al., 2003). Another well known inducer of DNA damage is cigarette smoke, which causes oxidative DNA damage and creates adducts in the DNA of cells. Hereby smoking is the primary cause of lung cancer in the world (Jackson and Bartek, 2009).

In normal circumstances the DNA is also being damaged. UV light from the sun can form up to ten thousand intrastrand crosslinks per cell per hour, however these damages are normally repaired very efficiently through different mechanisms in the cell (Jackson and Bartek, 2009; Luijsterburg and van Attikum, 2011)

While all DNA defects are hazardous for the cell, double-stranded breaks (DSBs) are particularly threatening when they are unresolved or inaccurately repaired. DSBs are the cause of severe genotoxic stress, and incorrectly repaired DSBs can result into genomic rearrangements. The latter may result in loss of genetic information, mutations or chromosomal translocations that lead to cancer. DSBs arise also after use of various cancer drugs, for example topoisomerase inhibitors, or are naturally generated during meiotic recombination (Van Gent et al., 2001; Ciccia and Elledge, 2010).

Repair of DSBs is accomplished through two main mechanisms, namely homologous repair (HR) and non-homologous end joining (NHEJ). Whereas HR restores the damage without losing genetic information by using the sister chromatid as a template, NHEJ is more prone to generate errors since it will simply join the two broken DNA ends together without use the of a correct template (Kanaar et al., 2008). After the formation of a DSB, besides DNA repair also a DNA damage signaling response is activated, known as the DNA damage response (DDR). The DDR is an elaborate signal transduction cascade that is capable of sensing DNA damage and translates this signal into various cellular responses. These include DNA repair pathways but also activate the DNA damage checkpoint. Activation of the checkpoint results in a transient cell cycle arrest that provides time for repair to take place. The DDR prevents progression of damaged DNA into mitosis and thereby avoids the propagation of mutated and/or damaged DNA to the daughter cells. Ultimately, if the damage remains unresolved, the DDR ensures the cell stops proliferating by inducing apoptosis or senescence (Bartek and Lukas, 2007; Misteli and Soutoglou, 2009).

Activation of the DDR is a complicated process which involves the accumulation of a variety of DDR factors. Specific DDR factors are recruited on the chromatin at the site of the lesion and this localization is a crucial step for activation of the DDR. After DNA damage, the chromatin around the sites of damage functions as a scaffold for DDR factors, thereby regulating their recruitment. Next, this signal is amplified by recruiting more DDR proteins and spreading the accumulation across the chromatin. The DDR signal is then subsequently transduced from the site of damage throughout the nucleus where target proteins further activate the DNA damage checkpoint. In this review, I will discuss why the spatial-temporal organization of the DDR is crucial for its activation and how it influences the activation of specific components of the DDR. The focus of this review will therefore lie on how DDR proteins are organized into DNA damage-induced foci and how these foci can be artificially recreated to study the DDR. This approach presents the DDR as a dynamic signaling cascade, which plays not only a role in DNA repair but also influences cell fate. As the DDR plays an important role in guarding malignant transformation, knowledge about the DDR could provide more insight into how the DDR can guide premalignant lesions into senescence and thereby prevent the development of cancer.

Spatio-temporal dynamics of the DDR – focus on focus formation

Formation and repair of double stranded breaks through HR and NHEJ

DSBs are generated during ionizing-irradiation (IR), chemicals, free-radicals or at stalled DNA replication forks. Additionally, DSBs also occur naturally in the meiotic recombination or in

recombination events necessary for the generation of unique genes required for a functioning immune system, for example the T cell receptor (Van Gent et al., 2001). Other damaging agents induce DSBs are various cancer drugs. Agents such as cisplatin or nitrogen mustard promote intrastrand crosslinks or interstrand crosslinks by inducing covalent bindings between DNA strands. Inhibition of topoisomerase I or II by topisomerase inhibitors, such as etoposide, also induce SSBs or DSBs. These agents stabilize the covalent DNA-topoisomerase complexes (Ciccia and Elledge, 2010).

DSBs are resolved either through NHEJ or HR. However, these two pathways function at different time points because for HR a sister chromatid is required to serve as a homologous template. These sister chromatids are only present in S and G2 phases of the cell cycle. In contrast to HR, NHEJ does not require a sister chromatid and therefore functions in all phases of the cell cycle (Polo and Jackson, 2011a).

During NHEJ, the DSB is first recognized by the Ku80-Ku70 heterodimer. Next, the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) is recruited to the lesion and this results in activation of its kinase activity. Subsequently, Artemis, XRCC4, XLF and DNA ligase IV are recruited which results in the ligation of the DNA ends and subsequent repair of the DSB (Figure 1). Because a DSB rarely consists of clean ends, these ends are under the continuous influence of nucleases and polymerases that trim the end (Wyman et al., 2008; Warmerdam and Kanaar, 2010). Therefore, this form of repair is more error-prone because it often leads to insertions or deletions (Kanaar et al., 2008; Polo and Jackson, 2011a).

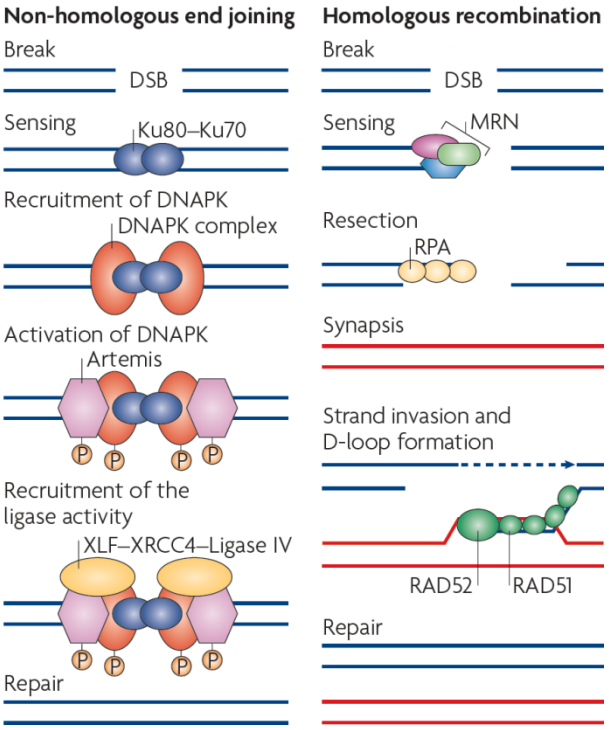

Figure 1 Non-homologous end joining and Homologous recombination
At a DSB, NHEJ is initiated by recognition of the break through Ku80-Ku70 and DNA-pk. With the help of nucleases, polymerases and ligases, the blunt DNA ends are put back together. In the case of HR, recognition is through the MRN complex and this leads to end resection and the strands are covered by RPA. With help of Rad51-Rad52 and other proteins, the sister chromatid is found and the DSB is restored without loss of genetic information. Adapted from Cimprich and Cortez, 2008.

Repair through HR begins with sensing the lesion through the MRN (Mre11-Rad50-Nbs1) complex. The endonuclease activity of this complex generates single stranded DNA (ssDNA). ssDNA are rapidly bound by replication protein A (RPA) and subsequently replaced by Rad51. Rad51 searches for the sister chromatid, followed by strand invasion with the help of Rad52 and proteins like FANCD1 and BRCA2. Strand invasion results in a Holliday junction, which later on, with help of DNA polymerases, helicases, ligases and topoisomerases gets resolved and thereby the DSB is repaired in an error-free manner (Figure 1) (Kanaar et al., 2008; Misteli and Soutoglou, 2009).

Recognizing DNA damage and activation of ATM/ATR

The proteins responsible for the DDR are classified in the following categories: sensors, transducers, mediators and effectors. Therefore, the first step in initiating a response is recognition of a DSB by sensors like the MRN complex and ssDNA binding protein RPA. The sensors subsequently activate the

transducer kinases, namely ATM, ATR and DNA-PK , which are members of the 'PI3K-like protein kinases' (PIKKs). While ATM is activated by MRN, the activation of ATR is through ssDNA regions, which are the result of processed DSBs (Shiloh, 2003; Luijsterburg and van Attikum, 2011).

Full activation of ATM is achieved by ATM autophosphorylation on serine 1981 (S1981) and this occurs simultaneously with its transition from an inactive dimer to an active monomer (Bakkenist and Kastan, 2003). Monomerization of ATM results in direct interaction with the MRN subunit Nbs1, thereby binding ATM effectively to the site of damage. In addition, the endonuclease activity of Mre11 is also required for full activation of ATM (Lavin, 2007). Active ATM now phosphorylates the C-terminal tail of histone H2AX (γH2AX). Next, the DNA damage checkpoint protein-1 (MDC1) is required through γH2AX. This ultimately results in spreading of γH2AX and more recruitment of MRN and ATM to the lesion (Figure 2) (Ciccia and Elledge, 2010; Warmerdam and Kanaar, 2010).

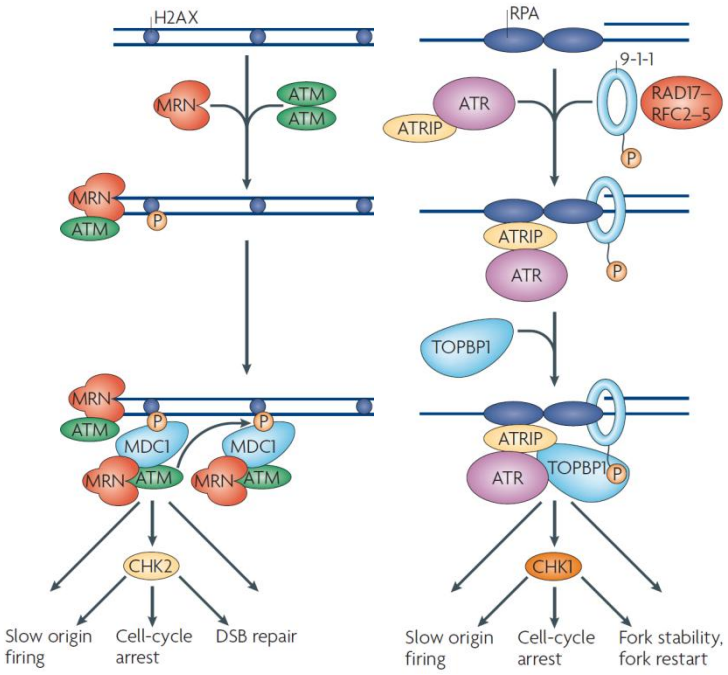

Figure 2 ATM and ATR signaling
The MRN complex senses a DSB and this results in the recruitment of activated ATM. ATM is further activated by by MRN and phosphorylates histone H2AX. MCD1 now binds γH2AX and recruits more ATM and MRN complexes. ATR-ATRIP is required the DSB after exposure of ssDNA covered by RPA. Activation of ATR through 9-1-1 complex and TopBP1 leads to Chk1 activation (Cimprich and Cortez, 2008).

ATR is activated in response to RPA-coated ssDNA. ATR is recruited to the DNA damage site by its binding partner ATRIP (Zou and Elledge, 2003). For complete ATR activation, a number of other proteins have to be loaded onto the DNA. The first step is the loading of the Rad9–Rad1–Hus1 (9-1-1) complex. The 9-1-1 complex is similar to the replicative sliding clamp PCNA, and is loaded onto the chromatin by the Rad17-RFC clamp loader complex (Parrilla-Castellar et al., 2004). In mammalian cells, the 9-1-1 complex recruits the essential mediator protein topoisomerase-binding protein-1 (TopBP1) to the chromatin (Delacroix et al., 2007). Once TopBP1 recruited the damage site by 9-1-1 it activates ATR (Cimprich and Cortez, 2008). ATR activation results in further ownstream signaling. ATR phosphorylates a variety of substrates, most notably Chk1. ATR-mediated Chk1 phosphorylation is regulated by the mediator protein Claspin, which is required to bring ATR and Chk1 together. Moreover, phosphorylated Rad17 is also required for the interaction with Claspin, which then results into the phosphorylation of Chk1 by ATR (Figure 3) (Cimprich and Cortez, 2008). In summary, with help of a group of mediators, including TopBP1, 53BP1, MDC1 and BRCA1, transducer proteins ATM and ATR are capable of activating effector proteins like Chk1 and Chk2. Chk1 and Chk2 subsequently spread the damage signal from the sites of damage throughout the nucleus to further induce the DNA damage checkpoint (Figure 3) (Bartek and Lukas, 2003).

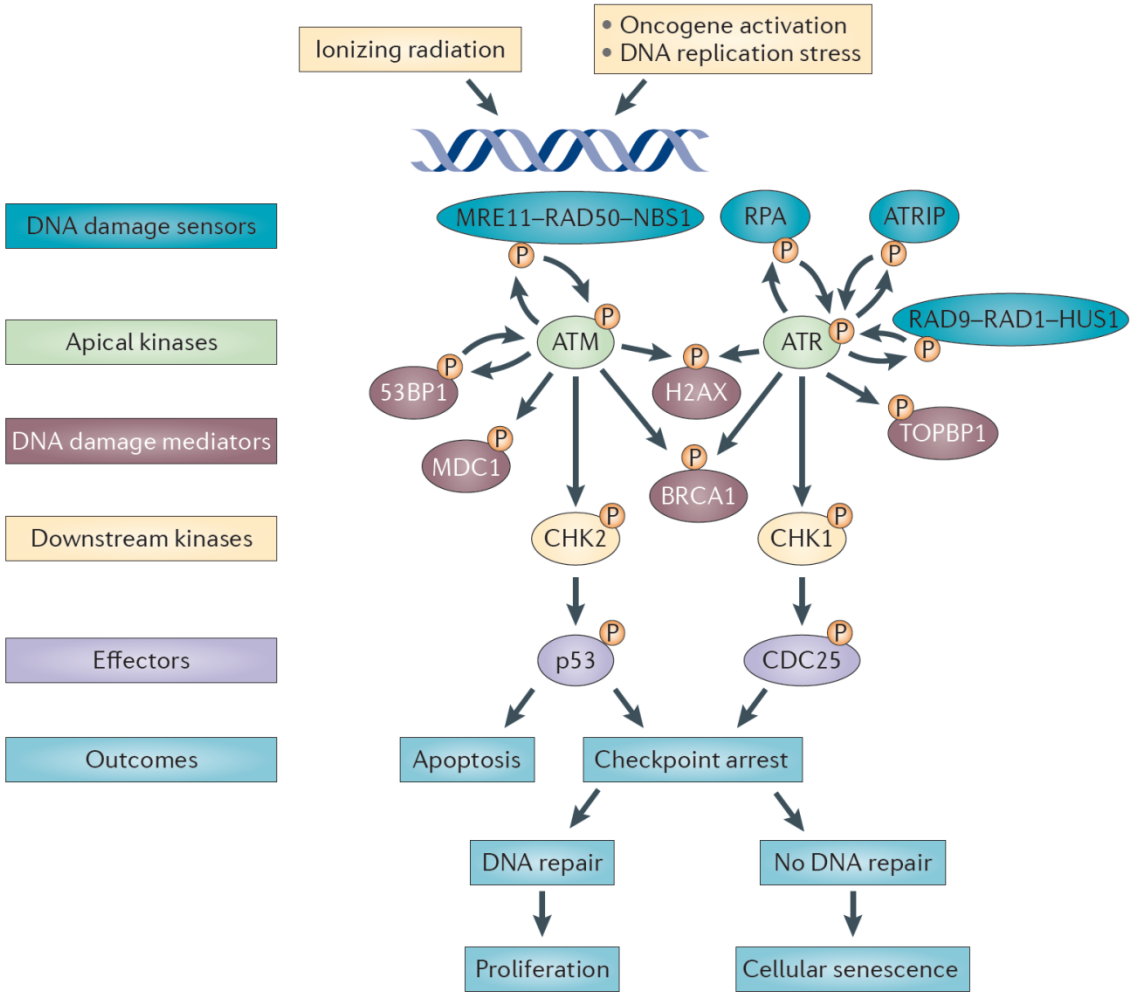

Figure 3 Overview of the DDR
A DNA lesion is detected by the sensor complex MRN. This leads to activation of ATM or activation of ATR after the formation of ssDNA. Through mediator activity the downstream kinases Chk1 and Chk2 are activated which can phosphorylate several effector proteins. This leads to DNA repair, cell cycle arrest or senescence (Sulli et al., 2012).

DNA damage-induced focus formation

A hallmark of DDR signaling is the accumulation of DDR proteins to site of the lesion. This requires a complex spatial and temporal coordination of these DDR factors. Over the years, microscopy based research has given much more insight into how these proteins assemble and disassemble at these so called DNA damage-induced foci. The foci, also called IRIF, IR-induced foci, represent DDR activation at the site of damage. Foci function as a platform for hundreds to thousands of repair proteins. Creating these high concentrations of proteins increases the efficiency of the DNA damage response and repair. However, the functionality of this massive accumulation is not fully understood (Lisby and Rothstein, 2009).

A wide variety of damage proteins are present in DNA damage-induced foci. These either bind directly to the lesion or the chromatin, for example the MRN complex, RPA or MDC1 while others bind via protein-protein interactions, for example ATM, ATR, TopBP1 or 53BP1 (Bekker-Jensen et al., 2006). Even though the foci first form at the site of damage, they spread along the chromatin

away from the lesion. During this event, the chromatin functions as a scaffold for the recruitment of the DNA repair proteins. As a consequence, the chromatin is modified by post-translational modifications like phosphorylation and/or ubiquitination. Through these modifications, other DDR factors start to interact with proteins of the DNA in the foci (Bekker-Jensen and Mailand, 2010).

In the case of HR, also a smaller subset of foci are observed. In this case, the RPA-coated ssDNA at the lesion functions as a scaffold instead of dsDNA surrounding the lesion. This initiates ATR signaling and also attracts other proteins involved in homologous repair such as Rad51, Rad52, BRCA2 and FANCD2. Other DDR factors required for the recognition of these foci are the 9-1-1 complex and other factors required for checkpoint activation, like ATRIP and TopBP1 (Figure 4) (Bekker-Jensen et al., 2006). Because HR requires the presence of a sister chromatid, these foci can only be visualized after DNA replication, during S and G2 phase. The proteins involved in NHEJ do not associate in foci, possibly because they are present in low concentrations at the DSB (Misteli and Soutoglou, 2009).

Once formed, these foci are not static structures. The exchange of proteins from the damaged chromatin and the freely diffusing proteins is highly dynamic. The time a protein resides on the chromatin is determined by their function on the chromatin (Soutoglou, 2008). However, not all DDR factors are accumulating in foci in a manner that can be visualized. For example, is activated after DNA damage on the chromatin but does not localize at foci (Smits, 2006; Smits et al., 2006; Warmerdam et al., 2010). Instead of concentrating at the site of damage, Chk1 swiftly spreads throughout the nucleus after it becomes phosphorylated at the site of damage. Because the activation of Chk1 is through ATR, this shows that Chk1 transduces the signal from the site of damage to other parts of the nucleus (Lukas et al., 2003; Bekker-Jensen et al., 2006).

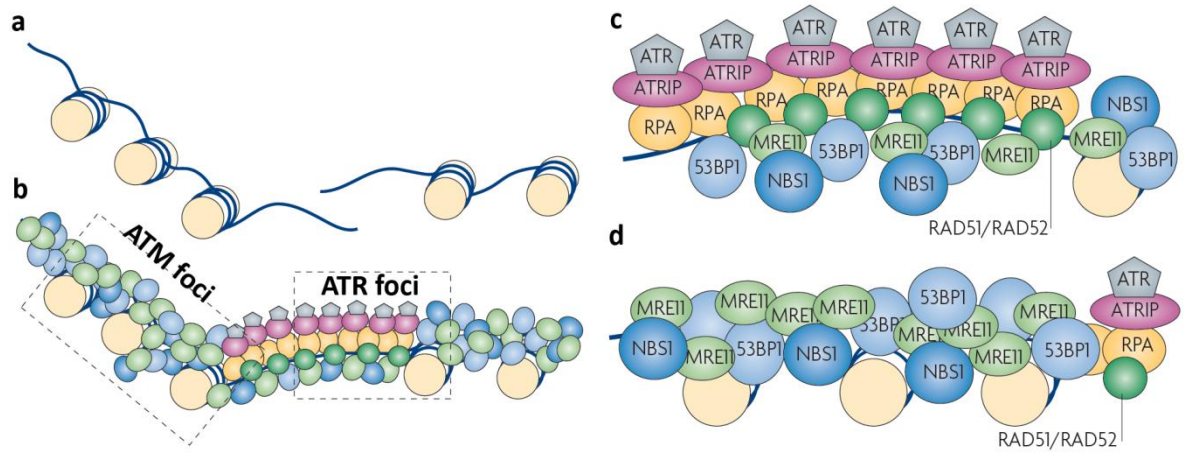

Figure 4 Schematic representation of a focus at a DSB
A. Nucleosomes with a DSB. B. A repair focus with in the centre a microfocus containing proteins bound by ssDNA and flanked by proteins binding the surrounding chromatin. C. Microfocus at the site of resected DNA. ssDNA is bound by RPA and focus formation is followed by accumulation of ATR-ATRIP, MRN complex and recombination factors Rad51 and Rad52. D. Flanking chromatin is covered by the MRN complex, MDC1 and BRCA1 and 53BP1. These proteins spread several kilobases away from the break site. Note that not all proteins are visualized in this representation. Adapted from Misteli and Soutoglou, 2009.

Signal amplification spreads the signal into the surrounding chromatin

After detection of a DSB, the signal spreads from the lesion into the surrounding chromatin up to a megabase from the original DNA lesion, thereby resulting in amplification of the signal (Bekker-Jensen et al., 2006). The first step in the amplification is the formation of γH2AX at the site of the DNA lesion and at the neighboring chromatin. Phosphorylation of H2AX on serine 139 is performed by ATM, ATR or DNA-PK and this creates γH2AX (Fernandez-Capetillo et al., 2004). The phosphorylation of H2AX enables MDC1 to initially recognize the γH2AX adjacent to the lesion, to which it binds through its BRCA1 C-terminal (BRCT) phospho-recognition domain. After MDC1 is activated and bound to γH2AX, it binds active ATM to the chromatin through MRN complex component Nbs1 (Figure 4D) (Stucki et al., 2005; Wu et al., 2008; Ciccia and Elledge, 2010; Polo and Jackson, 2011a). This assembly is very swift and the maximal accumulation of these factors into foci is established within a few minutes after exposure to damaging agents (Figure 5A) (Bekker-Jensen and Mailand, 2010). After ATM is anchored to MDC1, ATM subsequently spreads the phosphorylation of H2AX to the neighbouring chromatin, resulting in amplification of the signal (Figure 5B). However, the distribution of γH2AX is not uniformly among the chromatin. Because phosphorylation of H2AX in distal regions does not require MDC1, this suggests that this is performed by ATM which is not bound to the chromatin by MDC1 and can therefore spread further away from the DSB (Savic et al., 2009).

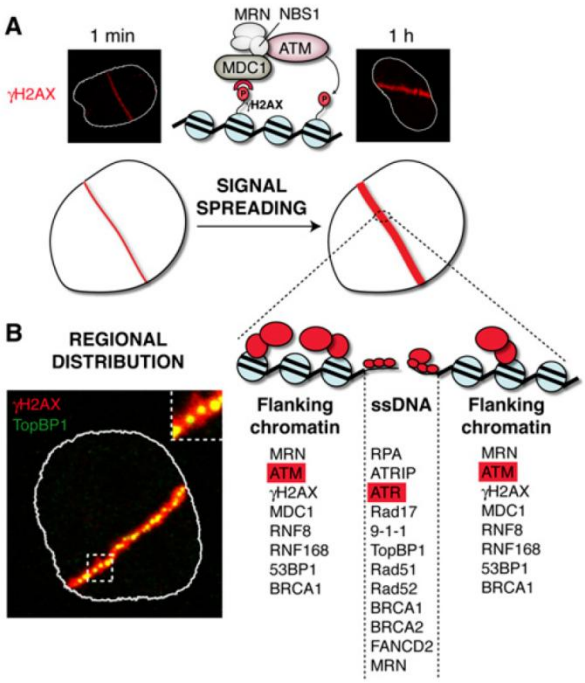

Figure 5 Amplification of the DDR signal
A. Quick formation of γH2AX after damage induction. Signal is spread by MDC1 anchoring to γH2AX and recruiting more active ATM. B. Distribution of proteins on the chromatin. TopBP1 is only found in microfoci which contain ssDNA. γH2AX shows how far the signal can spread into the surrounding chromatin. (Polo and Jackson, 2011b)

However, the presence of γH2AX is dispensable for the initial recognition of a DSB and therefore the spreading of γH2AX is proposed to be primarily responsible for the maintenance of the DDR signal. As a result, a two-stage model has been suggested for the recruitment of DDR factors to DSBs (Celeste et al., 2003). In this model, initial migration is performed independently of H2AX while the subsequent recruitment of factors to distal chromatin regions is dependent on γH2AX spreading. Therefore, in the early DNA damage response, ATM, ATR, 53BP1, BRCA and MRN are recruited to the chromatin without the presence of γH2AX (Yuan and Chen, 2010). However, interactions between γH2AX and MDC1, ATM and NBS1 generate a positive feedback loop that amplifies the H2AX signal and promotes additional recruitment of MDC1, ATM and the MRN complex, thereby further intensifying the signal (Lou et al., 2006). These additional proteins do not accumulate as fast as the initial recruitment-wave of MRN, MDC1 and γH2AX but follows after a short period of a few minutes (Figure 5A) (Bekker-Jensen and Mailand, 2010). Besides 53BP1 and BRCA1, also a variety of

chromatin modifying proteins, that affect acetylation, methylation, sumolyation and ubiquitination are attracted to the foci during this time. For example, p400 ATPase, which reduces nucleosome stability. Additionally, γH2AX bound MDC1 also recruits proteins like the histone acetyltransferase (HAT) TIP60 and the RING-domain E3 ubiquitin ligase RNF8. These proteins modulate the chromatin which stimulates ATM activity and also attracts other DDR factors like BRCA1 and 53BP1 (Lukas et al., 2011).

Additionally, after the establishment of ATM activation, HR is initiated and this leads to resection of DSB ends. This ultimately results in the loss of activated ATM and activation of ATR. ATR also phosphorylates γH2AX and therefore further induce the checkpoint amplification (Warmerdam and Kanaar, 2010). In conclusion, when the DSB is recognized and ATM and ATR are attracted to foci and subsequently activated, they phosphorylate a variety of mediator proteins and these further amplify the signal by bringing more ATM and ATR substrates to the site of damage (Zhou and Elledge, 2000).

Transducing the DNA damage signal downstream of ATM and ATR

When ATM and ATR are activated, this starts the next level of activation by attracting several mediator proteins to the foci. After the early formation of the foci, MDC1 attracts other mediators required to further transducer the DNA damage signal. By attracting proteins like BRCA1 and 53PB1, downstream checkpoint activation is promoted. Phosphorylation of MDC1 by ATM creates a binding site for the E3 ubiquitin-ligase RNF8. RNF8 subsequently ubiquitylates γH2A, this does not target the protein for degradation but promotes BRCA1 and 53BP1 recruitment to the DSB (Kolas et al., 2007; Al-Hakim et al., 2010). In contrast to BRCA1, 53BP1 does not have a ubiquitin binding domain but binds to methylated lysines, which are exposed upon ubiquitination, through its Tudor domain (Huen et al., 2007; Bekker-Jensen and Mailand, 2010; Ciccia and Elledge, 2010). Another DDR factor attracted in a RNF8 dependent matter is Rad18. Rad18 is involved in ubiquitination of proliferating cellular nuclear antigen (PCNA) after UV-induced DNA damage and involved in repair of DSBs after HR (Huang et al., 2009). Attraction of 53BP1 or BRCA1 determines which pathway is initiated since 53BP1 stimulates repair through NHEJ while BRCA1 promotes HR (Ciccia and Elledge, 2010). Between the substrates of both ATM and ATR there is overlap which indicates their crosstalk when activated.

After the recruitment of these proteins is established, ATM and ATR activate Chk2 and Chk1, respectively. Activation of Chk1 and Chk2 spreads the DNA damage signal throughout the nucleus. Both effector kinases are capable of phosphorylating CDC25A which results in the degradation of this phosphatase (Mailand et al., 2000). Loss of CDC25A G1/S phase subsequently leads to inactivation of several Cyclin Dependent Kinases (CDKs), thereby inducing a cell cycle arrest (Warmerdam and Kanaar, 2010). Chk2 is capable of phosphorylating p53. This phosphorylation stabilizes p53 and inhibits its degradation, so it can function as a transcription factor. The phosphorylation of both p53 and CDC25 may result in either apoptosis or a DNA damage-induced cell cycle arrest (Bartek and Lukas, 2003).

Nuclear architecture of DNA repair

The DDR factors are diffused in the nucleus and are rapidly recruited after the detection of a DNA lesion. In the nucleus, the chromatin is organized in either euchromatin or heterochromatin. Furthermore, the mammalian nucleus is also compartmentalized, for example transcription and replication are concentrated to specific regions. Due to the complex structure of the genome, the spatial organization of the genome also regulates the DDR (Misteli and Soutoglou, 2009).

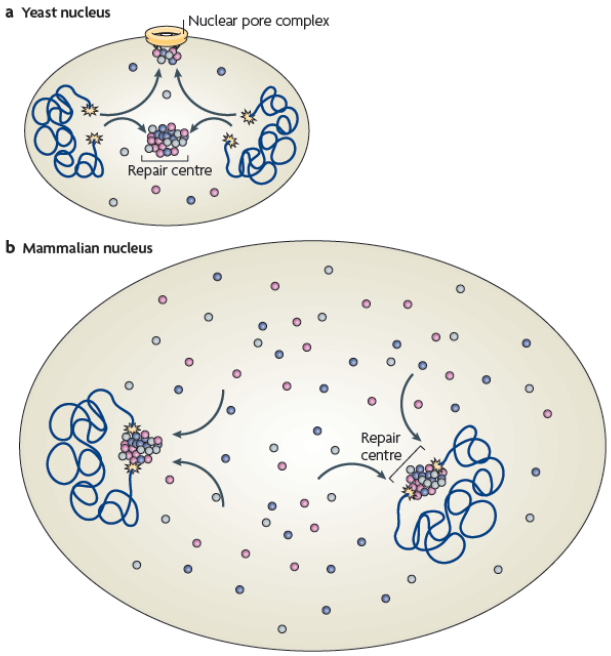

Figure 6 Nuclear architecture for DNA repair in yeast and mammals
A. In yeast, DSBs are migrated to a single repair centre or to the nuclear pore complex. B. In mammalian cells, the sheer size of the nucleus disables DSBs to migrate to one repair centre. Therefore, repair is performed in multiple repair centers (Misteli and Soutoglou, 2009).

In *S. cerevisiae,* DNA repair is compartmentalized into repair centers. After the formation of DSBs, specific repair foci are created which can repair multiple DSBs at the same time. Particularly, the formation of DSBs at two different sites in the nucleus finally resulted in their localization into a single repair foci (Lisby et al., 2003). Another example of relocalizing DSBs are irreparable DSBs. These irreparable DSBs are migrated to the nuclear periphery, specifically nuclear pores, where their repair is possibly facilitated (Figure 6A) (Nagai et al., 2008; Oza et al., 2009). However, in mammalian cells these principles do not apply. DSBs do not localize to repair centers and their mobility is limited (Figure 6B) (Soutoglou et al., 2007). This distinction is because yeast and mammalian cells have a different nuclear architecture. While chromatin can move similarly, the nuclei are so different in size that, for example, the distance for a DSB to migrate to a pore or find an excising DNA repair foci in a mammalian cell would simply be too great (Misteli and Soutoglou, 2009).

Another complication for efficient repair is the compaction DNA into heterochromatin. To initiate repair, the heterochromatin has to be made more accessible. This is performed by KAP1, which is phosphorylated by ATM. This phosphorylation event relaxes the compact state of the chromatin at the site of a break (Noon et al., 2010). Furthermore, the spreading of γH2AX is also under the influence of nuclear architecture. For example, it cannot disperse well on actively transcribed genes (Iacovoni et al., 2010). Moreover, heterochromatin is more resistant to γH2AX formation and when γH2AX is eventually formed in heterochromatin regions it remains persistent (Cowell et al., 2007; Goodarzi et al., 2010).

For more efficient DNA repair to take place, it is required to relax heterochromatin. However, even after relaxation, repair is still happening with slower kinetics than repair in euchromatic regions. Altogether, these findings nicely illustrate how nuclear architecture influences the dynamics of the DDR (Cann and Dellaire, 2011).

DNA damage signaling through artificial localization of DDR factors to chromatin

One of the hallmarks of the DDR is the accumulation of proteins at sites of DNA damage, observed in cells as nuclear foci. In recent years, a variety of DDR proteins have been discovered that localize into these foci after damage, however the functionality of their recruitment into these foci was still unclear. The importance of DNA damage-induced foci and the localization of proteins into these nuclear structures has recently been illustrated by two different studies. In *S. cerevisiae*, Bonilla et al. show how the localization of checkpoint proteins to the chromatin activates the DDR. Soutoglou et al. show that this mechanism is also presents in mammalian cells and therefore it seems highly

conserved. Besides, these studies show that the localization of checkpoint proteins to chromatin is important for the activation of both ATM and ATR.

In both studies, the bacterial Lac operator (LacO) sequence is used to artificially localize the proteins of interest to the chromatin. Multiple repeats of the LacO sequence are stably integrated into the genome, either in *S. cerevisiae* or NIH-3T3 cells derived from mice. The LacO sequence binds with high affinity to the Lac-repressor (LacR). By fusing a protein of interest to the LacR, the protein is quickly recruited to the LacO repeat. By additional coupling the LacR-fusion protein to a fluorescent GFP-like protein also makes it eligible for visualization in living cells. This system makes it possible to artificially localize proteins to the same position in the nucleus.

DDR without damage through ATR

In this study in *S. cerevisiae*, the induction of the DDR through ATR is recreated by expressing proteins Ddc1 (mammalian Rad9) and Ddc2 (mammalian ATRIP) fused to GFP-LacR. Through this approach, both Dcd2 and Ddc1 are localized to the LacO repeat.

To check whether the colocalization results in DNA lesions, the localization of Rad52 (also Rad52 in mammalian, but has a different role there), a downstream DNA repair protein is monitored. Rad52 does not localize to the lacO repeat, suggesting that the colocalization of Ddc1 and Ddc2 to the chromatin does not result in actual damage, like a DSB, and repair proteins are therefore not attracted to this site.

Rad53 (mammalian Chk2) phosphorylation is used as a readout for checkpoint activation. When Ddc1 and Ddc2 are both localized to the chromatin, this results in the phosphorylation of Rad53, indicating activation of the DNA damage checkpoint. Furthermore, Rad9 (mammalian 53BP1) is phosphorylated by Mec1 (mammalian ATR) and this is required for Rad53 activation. When Mec1-Ddc2 and Ddc1 are colocalized to the LacO sequence, this leads to the phosphorylation of Rad9.

To further confirm the induction of a DDR, a downstream target of the effector kinase Rad53 is investigated. In response to damage, Rad53 phosphorylates Dun1 which then subsequently phosphorylates Sml1. When Sml1 is phosphorylated, it is targeted for degradation. Because the presence of Dun1 could not be observed, Sml1 is used as a read out for RAd53 activity. Indeed, when Ddc1 and Ddc2 are colocalized, this leads to a decrease of Sml1 levels, indicating downstream signaling from Rad53.

A hallmark of DNA damage checkpoint activation is the induction of a G2/M cell cycle arrest. If Ddc1 and Ddc2 colocalization is able to elicit a DNA damage response, this colocalization should be able to activate the checkpoint and keep cells in a cell cycle arrest. To test this, cells are synchronized by arresting them in G2/M phase with nocodazole and after release from nocodazole, cells are treated with α factor to not keep them cycling beyond G1. The cells that do not have a LacO sequence entered G1 90 minutes after the removal of nocodazole. However, when Ddc1 and Ddc2 are both expressed in the presence of a LacO sequence, the cells remain in G2/M as shown by the presence of a 2N peak. This confirms that Ddc1 and Ddc2 association on chromatin is capable of inducing DNA damage dependent checkpoint activation without any actual DNA damage present.

Furthermore, the minimal amount of LacO repeats required for Rad53 phosphorylation is 40 LacO repeats and Rad53 phosphorylation increases with the amount of LacO sites. This suggests that the amount of recruited fusion proteins to the LacO sequence is directly correlated to the amount of Rad53 phosphorylation. Therefore, the amount of Ddc1 and Ddc2 determines to what extent Rad53 can be phosphorylated and thus, the threshold for DDR signaling is present at low concentrations of Ddc1 and Ddc2 on chromatin.

The requirements for checkpoint activation are the presence of both Ddc1 and Ddc2 on the chromatin, demonstrating their dependency on each other. Additionally, the dependency of other proteins for checkpoint activation was investigated. Mec1 and Rad9 are required for inducing checkpoint activation through Ddc1 and Ddc2 colocalization. In the absence of these two proteins, Rad53 phosphorylation does no longer take place. However, the presence of Rad24 (mammalian Rad17) is not required. This indicates that the clamp loader protein Rad24 is only required to recruit Ddc1 to the DNA, an action that is now redundant through LacR localization. Furthermore, the presence of the other two subunits of the 911 complex Mec3 (hHus1) and Rad17 (hRad1) is not necessary for inducing Rad53 phosphorylation.

This research shows that it is possible to bypass the need for actual DNA damage when inducing checkpoint activation. Checkpoint activation therefore relies on the colocalization of Mec1-Ddc2 and the 911 complex, particularly component Ddc1. Moreover, other DDR factors become dispensable, giving more insight into the choreography of the initiation of the response. Therefore, in this system, the DNA functions as a scaffold for the recruitment of DDR factors and the chromatin functions as mediator for signal amplification.

DDR without damage through ATM

Also in mammalian cells, Soutoglou and Misteli show that localization of DDR proteins to the chromatin is enough to trigger checkpoint activation. With a similar system, they show that localization of the MRN complex subunits, Nbs1 and Mre11, results in a checkpoint-mediated arrest without the induction of DNA damage. In this study, the LacO system is implemented in mouse NIH-3T3 cells.

To assess whether the localization of DDR factors to the chromatin can result in activation of DNA damage signaling, phosphorylation of H2AX at the LacO site is used as an indicator for active DDR signaling. Furthermore, to check for actual DNA damage, several control experiments were performed. However, none of these experiments indicate actual damage being present at the LacO site, excluding that the artificial localization a DDR factor to chromatin is causing lesions in the DNA. Moreover, in the presence of a LacO binding inhibitor, the overexpression of the DDR-LacR proteins does not lead to γH2AX formation, indicating that the crucial step in induction of a DDR is indeed localization.

Two components of the MRN complex, namely Nbs1 and Mre11, are the first factors investigated for their ability to induce a DDR through artificial localization. Overexpression of fusion proteins Nbs1-LacR or Mre11-LacR in the presence of a LacO sequence, results in their localization to the LacO site and subsequent phosphorylation of H2AX at the same site in 60 to 70% of the cells. Other markers for DDR activation are the phosphorylation of Nbs1 and ATM and the accumulation of cells in G2 phase. These observations all indicate that localization of MRN proteins to the chromatin induces DNA damage signaling, as observed through phosphorylation of H2AX, Nbs1 and ATM, and ultimately, checkpoint activation by inducing a cell cycle arrest.

In addition to the MRN complex, also other components of the DDR are investigated. MDC1 is capable of binding to H2AX and important for recruitment of downstream checkpoint and repair proteins, like 53BP1 and BRCA1 (Stewart et al., 2003). The expression of the MDC1-LacR fusion protein also leads to the formation of γH2AX foci at the LacO site, similar to the observation for Nbs1 and Mre11 localization. Additionally, cells arrested in G2 phase when MDC1 is immobilized, indicating checkpoint activation. An abrogated form of MDC1 without BRCT domains is incapable of binding γH2AX. Therefore, tethering this form of MDC1 to the chromatin does not lead to γH2AX

formation and hence, no checkpoint activation. The interaction between the BRCT domain and γH2AX is needed for a prolonged interaction and subsequent accumulation of DDR factors (Stucki et al., 2005). Without this domain, tethering MDC1 to the chromatin will not be able to bind γH2AX and induce the downstream effects of the DDR.

To transduce the signal from the lesion to the surrounding chromatin and throughout the nucleus, activation of ATM is required. Immobilizing a fragment of ATM, which includes the kinase domain, leads to γH2AX formation. Furthermore, ATM itself is phosphorylated and a cell cycle arrest is induced, indication checkpoint activation. This indicates the importance of ATM localizing to the chromatin to function in contrast to being freely diffused in the cell. However, the immobizilation of the downstream effector kinases Chk1 and Chk2 does not lead to DDR signaling. This is illustrated by the absense of both γH2AX and a cell cycle arrest. Possibly, these effector kinases are too far downstream into the signaling cascade to elicit a DNA damage signal by only locating them on the chromatin. Moreover, Chk1 and Chk2 are normally not present at DNA damage foci but spread throughout the nucleus after activation by ATM or ATR. This suggests that active ATM or ATR is required for Chk1 or Chk2 activation and not localization of these factors on the chromatin.

The sequential order and dependence of the DDR is not fully understood even though the immobilization of single factors leads to recruitment of other DDR factors for initiation of a full DNA damage response. The localization of Nbs1 to the chromatin results in recruitment of downstream proteins 53BP1, MDC1 and direct partner Mre11 (Figure 7A). Likewise, MDC1 also recruits these factors when bound to the chromatin. Interestingly, MDC1 is a factor downstream of the MRN complex, but it still is capable of inducing recruitment of upstream MRN factors, providing insight into how downstream factor are capable of amplifying the signal by also recruiting upstream factors. Furthermore, the abrogated form of MDC1, lacking the BRCT domain, still initiates mobilization of Nbs1 and Mre11 (Figure 7C). This indicates that binding of MDC1 to γH2AX is not required for attracting Nbs1 and Mre11 but supports the notion that the BRCT domain is needed for shielding γH2AX from phosphatases (Stucki et al., 2005). Locating a fragment of ATM to the DNA did only result in the recruitment of MDC1, likely to be attracted by its binding to γH2AX, showing that in this case MDC1 is not capable of attracting upstream factors for amplification (Figure 7D). Furthermore, immobilized Chk1 and Chk2 were also not able to recruit other factors.

These experiments have given more information about the hierarchy of the DNA damage response. While localizing Nbs1, Mre11 or MDC1 to the chromatin is sufficient for eliciting a DDR, indicated by H2AX phosphorylation, Nbs1, Mre11 and MDC1 are required by recruit one another to achieve this. This recruitment is not only necessary for a full response, but also it promotes the amplification of the signal on the chromatin. However, ATM is not in the same order as these proteins and only indirectly recruits one factor, MDC1, through H2AX phosphorylation. This suggests that only ATM and MDC1 are enough to amplify the signal without the presence of MRN components.

To further investigate this recruitment through γH2AX, $H2AX^{-/-}$ cells containing a LacO sequence were analyzed. When no H2AX is present, the recruitment of MDC1 and 53BP1 by MRN complex subunits

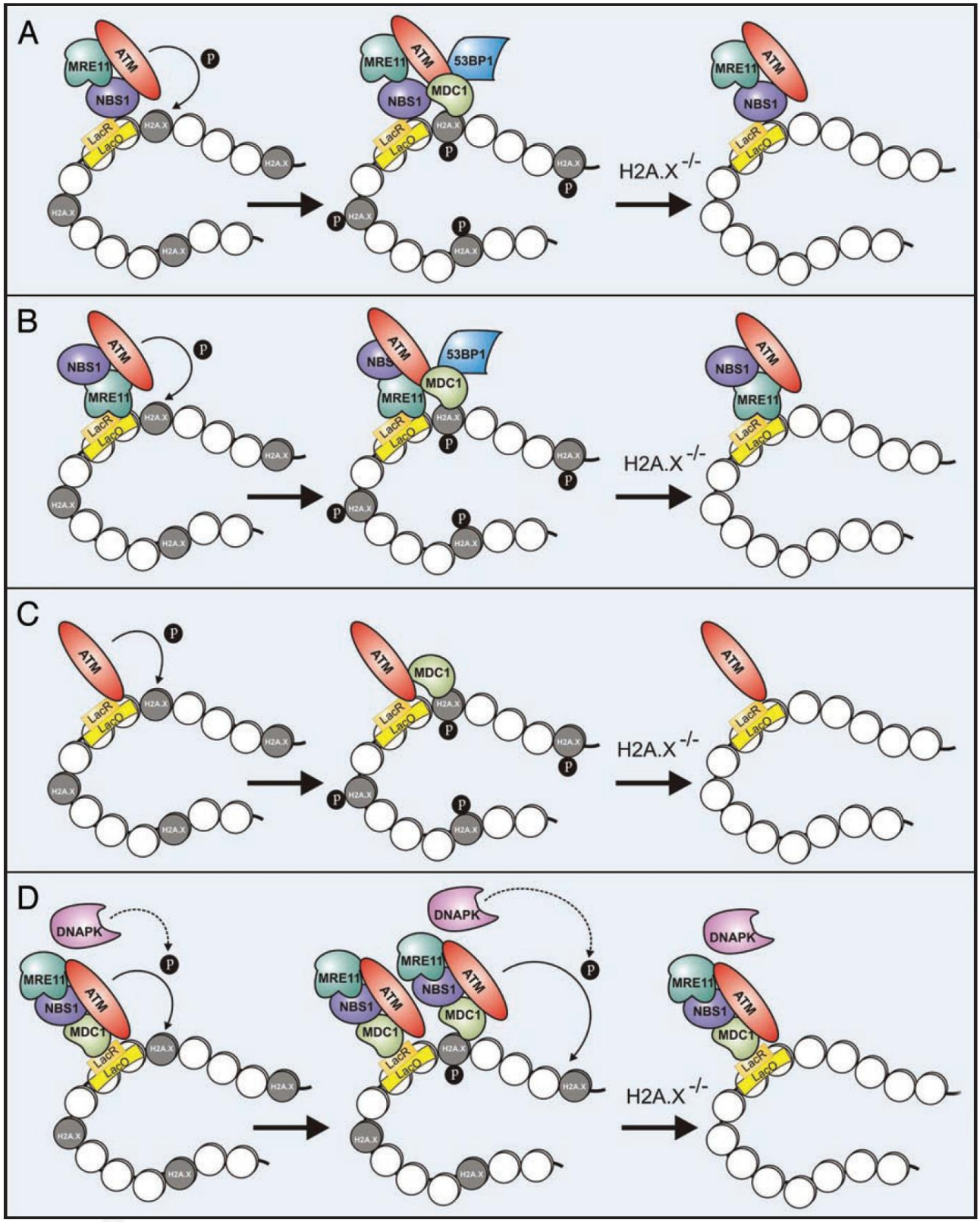

Figure 7 Localizing DDR factors artificially to the chromatin can initiate a DNA damage response without the presence of actual DNA damage

A. Nbs1-LacR can recruit Mre11 and ATM. This leads to the formation of yH2AX and attracts MDC1 and 53BP1. When yH2AX is absent, downstream DDR factors MDC1 and 53BP1 are no longer attracted and this abrogates signal amplification. B. Similar to Nbs1 tethering, Mre11 can also recruit Nbs1, ATM, MDC1 and 53BP1. Also in this case, downstream signal amplification is impaired. C. ATM (kinase domain)-LacR elicits a DNA damage response through phosphorylation of H2AX and attraction of MDC1. However, no other upstream factors are recruited. Furthermore, MDC1 can only be attracted by ATM in the presence of H2AX. D. MDC1-LacR can recruit ATM and DNA-pk which results in yH2AX formation. Also upstream factors Nbs1 and Mre11 are attracted, indicating signal amplification. In the absence of H2AX, MDC1 can no longer attract ATM but is still capable of recruiting Mre11 and Nbs1. Adapted from Soutoglou, 2008.

Nbs1 or Mre11 is strongly decreased. Likewise, recruitment of MDC1 by ATM is also impaired, confirming the requirement for γH2AX presence to recruit MDC1. Furthermore, absence of H2AX together with targeting DDR factors to the chromatin did not lead to a G2 phase arrest, suggesting they are indeed checkpoint defective. This demonstrates the important role of H2AX in the accumulation of multiple DDR factors to amplify the DDR signal and elicit a full DNA damage response.

These reports demonstrate that in yeast and mammalian cells, immobilizing DDR factors can result in checkpoint activation. This can be through localizing components of the 9-1-1 complex with ATR (Ddc1 and Ddc2 in yeast) or through the MRN sensor complex components Nbs1 or Mre11. By inducing γH2AX formation, the DNA damage response is activated. Also mediator protein MDC1 induces checkpoint activation and amplification by attracting upstream DDR factors Nbs1 and Mre11. This attraction does not take place when the ATM kinase domain is used, which only leads to recruitment of MDC1. In summary, the chromatin functions as a scaffold for the initiation of the DNA damage response. Localization of specific DDR factors are the key step in initiation of the DDR and this cancels the needs for an actual DNA lesion. This leads to the question of whether a response without damage is a mechanism actually present in cells. The induction of a DNA damage response could be beneficial for cells experiencing other signals than damage, but still want to elicit a cell cycle arrest or even apoptosis or senescence.

Persistent DDR signaling and senescence induction

When certain DNA damage proteins are tethered to one particular location on the chromatin, the DDR is initiated without an actual DNA lesion being present. This overrides the need of an actual DNA lesion, demonstrating that that initiation of the DDR is achieved by bringing the necessary proteins together on the chromatin. Overriding the need of a true lesion does not only happen with artificial localization, it has been described that during senescence a DNA damage response is activated without detectible DNA breaks (Pospelova et al., 2009). When DDR signaling is initiated without a DNA lesion, resolution of the signal could be difficult because repair is not required if there is no DNA damage. Therefore, this signaling could become persistent and could contribute to the long term effects of DDR signaling, for example senescence induction. However, it is still unclear if DDR activation without the presence of damage is a phenomenon that truly occurs in cells. To explore the possibility and potential implications of this phenomenon in more detail, the link between DNA damage signaling and senescence will be further discussed on the basis of recent literature.

Senescence is a state in which the cell has lost its proliferative capacity. The cell is permanently arrested and can therefore no longer enter the cell cycle. DNA damaging agents or telomere shortening trigger the induction of senescence (Campisi and d'Adda di Fagagna, 2007). In addition, senescence can be a consequence of oncogene activation, called oncogene-induced senescence (OIS) (Sulli et al., 2012). In the recent years, it has been discovered that DDR signaling plays an important role in establishing senescence. This research generally indicates DDR signaling in the presence of DNA breaks while Pospelova et al. show that the during senescence induction the DDR is activated without detectible DNA breaks. The link between senescence and the DDR was first illustrated by the accumulation of γH2AX foci in senescent cells during ageing (Sedelnikova et al., 2004). In addition, in the case of oncogene-induced senescence, the activation of the DDR proved to be of vital importance (Bartkova et al., 2006).

Activation of an oncogene can result in oncogene-induced replication stress. This is the result of collapsed replication forks or double stranded breaks due to enhanced replication speed (López-Contreras and Fernandez-Capetillo, 2010). In order to prevent oncogene-induced replication stress in cells, senescence is induced. The induction of senescence is dependent on DNA damage signaling since loss of ATM cells does not result into senescence after the overexpression of an oncogene like cdc6 or cyclin E (Bartkova et al., 2005, 2006). Additionally, in the absence of ATM or Chk2, expression of RasV12 does not lead to senescence and cells continue to proliferate (Di Micco et al., 2006). How exactly DDR signaling induces senescence was illustrated by generating cells which express highly active ATR. In response to DNA damage, TopBP1 normally activates ATR. Through the exogenous expression of an inducible ATR activating domain of TopBP1, cells were generated with overactive ATR signaling. Hyper activating ATR resulted in the phosphorylation of a variety of ATR targets, for example H2AX, SMC1 and Rad17. Interestingly, 72 hours after the activation of ATR cells started to become senescent. For example, observed by the formation of senescence-associated heterochromatin foci (SAHF) (Toledo et al., 2008). Interestingly, the appearance of SAHF in normal respiratory epithelium is only observed when these cells were challenged with the activation of an oncogene and not after treatment with DNA damaging agents. Indicating that SAHF formation could be a consequence of oncogene induced replication damage. Besides, SAHF seems to restrict the formation of DNA damage foci at that particular position on the chromatin. This is supported by the fact that perturbation of heterochromatin formation leads to an increase in γH2AX foci and DDR signaling. As a consequence of this increased DDR signaling cells undergo apoptosis (Di Micco et al., 2011). So while DNA damage signaling can induce senescence, oncogene-induced senescence can inhibit DNA damage signaling through formation of SAHF and this may be unfavorable in the resistance to malignant transformations.

Senescence has also been associated with the presence of persistent DNA damage foci at telomeres as shown by Fumagalli *et al.*. Irradiation of quiescent fibroblasts leads to the formation of many DNA damage foci. However, not all DNA lesions can be repaired and the presence of persistent DNA damage foci is strongly correlated to senescence. Additionally, inhibition of ATM resulted in an escape from senescence and cells started to proliferate again. Indicating that the induction of senescence dependents on ATM-mediated signaling. Interestingly, the nuclear localization of these persistent foci was significantly associated with telomeres (Figure 8) (Fumagalli et al., 2012; Hewitt et al., 2012). The introduction of a single DSB in a telomeric region in *S. cerevisiae*, resulted in the formation of a persistent foci. Ectopic localization of the telomeric protein TRF2 within the vicinity of a DSB suppresses DNA repair, resulting in an unrepaired focus and cell cycle arrest. However, it remains unclear if the persistent foci are actually responsible for the induction of senescence. Moreover, the inability of the DNA damage machinery to repair these types of telomeric lesions is not correlated to the shortening of telomeres, since this phenomenon also takes place in aged terminally differentiated cells which have long telomeres (Fumagalli et al., 2012). Taken together, these results suggest that DNA damage accumulates at telomeres during ageing and that due to the irreparable nature of these lesions, this persistent signaling induces cellular senescence. However, it remains to be shown if inducing telomeric damage alone is enough to force cells into senescence.

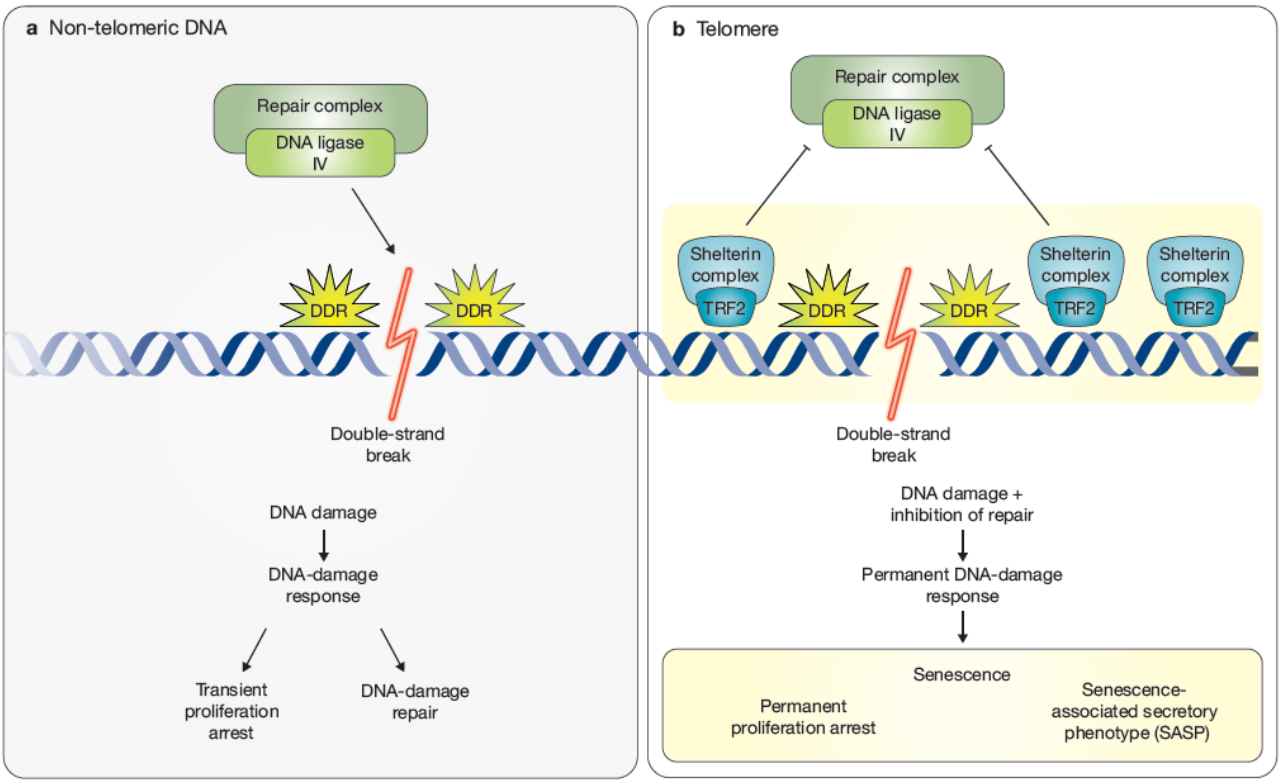

Figure 8 Telomeric damage results in persistent DDR signaling
The proteins sheltering the exposed ends of telomeres have the ability to suppress DNA damage repair. Therefore, a DSB results in non-telomeric DNA can result in a transient cell cycle arrest while in telomeric DNA this results in persistent signaling of the DDR without resolution through repair. This signaling results in a permanent DNA damage response which ultimately leads to senescence. Adapted from Van Tuyn and Adams, 2012.

However, in contrast to Fumagalli et al., another report indicates that there is no association of persistent DSBs to telomeres. These non-telomeric persistent DSBs do accumulate Heterochromatin Protein 1 (HP1), which indicates the formation of SAHF (Noda et al., 2012). Because SAHF also repel DNA damage repair, this suggests that both telomeric damage and irreparable DSBs which induce SAHF are capable of inducing persistent DNA damage signaling which can lead to senescence.

Another characteristic of senescence is hyper-activation of the cell due to over activation of the mTOR pathway. mTOR belongs to the PI3K-like family, as does ATM/ATR and DNA-pk. For this reason it is suggested that relevant pathways, like the ATM/ATR DNA damage response pathway, are also overactive in senescence. As a consequence, this could activate the DDR without actual DNA lesions being present during senescence. To investigate this, non-damaging methods were used to induce senescence in cells, namely HDAC inhibitors, sodium butyrate which induces senescence through stabilization of β-catening and downregulation of E2F in E1A and Ras-transformed rodent cells. Another method for inducing senescence is overexpressing p21 and p16, this causes a rapid cell cycle arrest and after a few days senescence is induced. Both senescence-inducing treatments result in an increase of γH2AX foci but without any detectable DNA breaks. The amount of γH2AX in these senescent cells was similar to the amount observed in irradiated cells. Notably, the γH2AX foci-formation in cells that finally become senescent was preceded by cell cycle arrest, indicating this γH2AX formation is not an early step in senescence induction. Furthermore, even though phosphorylated ATM was present, this was distributed throughout the nucleus and phosphorylated ATM or 53BP1 were not localized at the γH2AX foci, differentiating these foci from those observed after irradiation (Pospelova et al., 2009). This study shows that an altered type of DNA damage

response is required to induce or remain in the senescent state. This DDR is activated without the actual presence of DNA damage, but is most likely required to keep the cell in the arrested state.

Pospelova *et al.* show γH2AX foci-formation during senescence, indicating the presence of DNA damage signaling during this cell state. Initiation of this signaling does not begin with ATM phosphorylation because this phosphorylation is preceded by γH2AX foci-formation. Therefore the responsible kinase for γH2AX formation could be ATR, which involvement in senescence was also shown previously (Toledo et al., 2008). However, how activation of ATR without damage would occur is still unclear.

Furthermore, activating the DDR through damaging agents did not induce senescence. Together with the timing of the γH2AX foci formation this suggests that DDR activation is a later step in senescence induction. Furthermore, the observed absence of phosphorylated ATM at these foci indicates a difference between irradiation induced foci. Therefore it would be interesting to investigate how other DDR-associated proteins can localize to these γH2AX foci, for example Nbs1, Mre11 or MDC1. In a recent report it was observed that persistent foci formed after damage-induced senescence do not contain RPA and no DNA synthesis takes place, indicating that these lesions are not repaired. Furthermore, these foci accumulate active Chk2 and p53 (Rodier et al., 2011). Interestingly, an altered DDR response also occurs during mitosis (Giunta et al., 2010). Likewise, mitotic DDR foci do not contain 53BP1, but do contain Nbs1 and MDC1 for example. Moreover, the phosphorylation of Chk1 and Chk2 are also reduced in mitotic cells compared to cells in interphase. These results indicate that the DDR is a dynamic signaling cascade that depending on its role can activate specific components in order to control the cell cycle.

Future directions

Bonilla *et al.* and Soutoglou *et al.* have shown the minimal requirements for the initiation of a DDR. Besides, these studies show that these requirements are highly conserved throughout evolution since they seem to be very similar in both yeast and mammalian cells.The crucial step in activation of the DDR in cells is the localization of certain DDR factors to the same position on the chromatin. The proteins that are necessary for checkpoint activation are subunits of the sensor MRN complex and 9-1-1 complex (Nbs1, Mre11 and Ddc1 (in combination with Ddc2)), the transducer kinases (ATM and ATR) and the mediators (MDC1). Most likely the other proteins that have been previously been associated with checkpoint activation like, γH2AX, 53BP1, BRCA1 and TopBP1 either have a role in the recruitment of these basic factors or mediate amplification of the checkpoint signal. This also indicates that the regulation of chromatin is of crucial importance for an efficient DDR, since it is both a template for repair as well as the platform for the recruitment of DDR proteins. It will be interesting to uncover precisely which types of chromatin modulations are required for the DDR in future research.

However, DDR activation does not always require a damaged template. The localization of DDR factors to chromatin results in persistent DDR foci which consequently induce a senescence state. This indicates that the DDR has both different functions as well as different ways to get activated. Moreover, the role of proteins involved in the DDR seem rely on this difference in function, indicating that the DDR is a dynamic signaling cascade. This dynamic nature might be important for checkpoint timing and DDR foci regulation. Since, at specific moments in time or at specific sites in the cell, the DDR is repelled. For example, when cells enter mitosis, the full activation of the DDR is restricted to inhibit DNA repair. This is due to the exclusion of factors like 53BP1 and

RNF8 from DNA damage-induced foci, which impairs the full activation of the DDR. Not only can factors be repelled from foci during mitosis. Also heterochromatin, and more specifically SAHF, can inhibit the induction of the DDR through repelling γH2AX foci formation. This could be a tool for malignant cells to suppress the DDR. However, in contrast to SAHF, mitotic cells can form γH2AX foci, suggesting that the mechanism of repulsion of the DDR is different. Also, irreparable DSBs from similar foci to other IRIF but repair is resisted due to SAHF formation. Furthermore, telomeres are very effective in inhibiting DNA repair, resulting in persistent signaling which can induce senescence. This shows that an ongoing DNA damage response is coupled to ageing and senescence, possibly through two different types of persistent signaling, i.e. non-telomeric versus telomeric damage. The manner in which persistent DNA damage signaling without damage functions, could be very similar to the two mechanisms of persistent signaling in the presence of damage. Because the latter both repel repair and the other does not require repair, there is no resolution of the damage and this consequently leads to perseverance of the signal and senescence induction.

Interestingly, the formation of γH2AX foci seems to be a consequence of senescence rather than a factor for its initiation. However, not much is known yet about this exactly functions. Why would cells induce γH2AX foci-formation without the presence of DNA damage? Does the phosphorylation of H2AX in this case just support the induction of senescence? Also, how are cells able to initiate γH2AX foci-formation? Even though ATM is essential for the induction of senescence it does not seem to be involved in γH2AX formation in non-damage induced senescence. Moreover, what is the exact function of this signaling cascade? Since the initiation of DNA repair would be unfavorable and a cell cycle arrest has already been established. Perhaps, the accumulation active Chk2 and p53 at persistent foci can give more insight into the downstream signaling of these foci differentiates form transient foci. The persistent response could only be for checkpoint activation while repair is not initiated or unresolved due to the different nature of the formed foci.

To conclude, the functioning of the DDR is not only important for repairing DNA damage and inhibiting malignant development, dysfunctional DDR can also influence cell fate. Without proper ATM or ATR signaling, cells cannot enter senescence. Furthermore, defects rising in the DDR after oncogene-induced senescence can consequently reverse this state and cell can again proliferate. Therefore, persistent signaling or no lesion signaling can be important in the maintenance of cell fate. This could be the method for damaged telomeres to induce senescence and for transformed cells to further promote their senescent induction. How this is achieved is through the differential spatio-temporal regulation of foci formation and transduction of DDR signaling. Mutations in DDR signaling are quite common in malignant cells. However, while repair proteins can be affected, this shows that affecting only signaling also has a consequence on cell fate. Targeting therapy to restore checkpoint activation while repair is still dysfunctional could also provide a mechanism for again directing cells towards a post-replicate cell fate. A better understanding of these phenomena can give further insight into how DNA damage signaling, cancer and ageing are connected. This could provide new strategies in how the protective potency of DNA damage signaling can be used to our advantage in treating cancer.

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