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Double-strand breaks: signaling pathways and repair mechanisms

T. C. Karagiannis[a] and A. El-Osta[b,*]

[a] Molecular Radiation Biology, Trescowthick Research Laboratories, Peter MacCallum Cancer Centre, Melbourne (Australia), e-mail: tom.karagiannis@petermac.org
[b] The Alfred Medical Research and Education Precinct (AMREP), Baker Medical Research Institute, Epigenetics in Human Health and Disease Laboratory, Second Floor, Commercial Road, Prahran, Victoria 3181 (Australia), Fax: +61 3 8532 1100, e-mail: assam.el-osta@baker.edu.au

Abstract. Double-strand breaks arise frequently in the course of endogenous – normal and pathological – cellular DNA metabolism or can result from exogenous agents such as ionizing radiation. It is generally accepted that these lesions represent one of the most severe types of DNA damage with respect to preservation of genomic integrity. Therefore, cells have evolved complex mechanisms that include cell-cycle arrest, activation of various genes, including those associated with DNA repair, and in certain cases induction of the apoptotic pathway to respond to double-strand breaks. In this review we discuss recent progress in our understanding of cellular responses to DNA double-strand breaks. In addition to an analysis of the current paradigms of detection, signaling and repair, insights into the significance of chromatin remodeling in the double-strand break-response pathways are provided.

Key words. DNA double-strand breaks; signal transduction; trancriptional responses; homologous recombination; non-homologous end-joining; chromatin remodeling.

Introduction

DNA double-strand breaks (DSBs) are the result of two simultaneous nicks in opposite strands of the DNA helix. A requirement for DSB formation is that the two nicks are in sufficiently close proximity to one another (<12 bp) that base pairing and chromatin structure are unable to maintain the broken DNA ends juxtaposed.
It is known that DSBs can be generated following exposure of cells to exogenous agents such as ionizing radiation, radiomimetic drugs and topoisomerase inhibitors [1–5]. In addition, so-called pathological DSBs can arise from endogenous processes. For example, ~1% of inhaled oxygen is converted into oxidative free radicals [reactive oxygen species (ROS)] such as hydrogen peroxide and superoxide anions that have the potential to cause DSBs [5, 6]. Furthermore, DSBs may occur when the replication machinery meets nicks or other types of lesions in the template DNA [4, 7], and as discussed further below, DSBs may result from incorrect resolution by normal DNA-metabolic proteins such as RAG1 and RAG2 [4, 8–10].
Although they represent a major danger to the stability of the genome (discussed below), DSBs are created intentionally by specific nucleases during regulated chromosomal rearrangements. A well-characterized example of this is V(D)J recombination, in which immunoglobulin (Ig) heavy chain and light chain variable region genes and T-cell-receptor genes are assembled from germline variable (V), diversity (D) and joining (J) sites in B- and T-lymphocytes, respectively [8–13]. These recombination pathways are initiated by DSBs that are generated at target loci by the site-specific RAG1 and RAG2 nuclease complex [8–13]. Furthermore, physiological DSBs are produced during meiosis to initiate recombination between homologous chromosomes and are also generated during Ig class-switch gene rearrangement pathways [4, 5].
It has been suggested that cells are able to adapt to low levels of irreparable DNA damage [4, 14]. However, only a single DNA DSB is potentially cytotoxic or under cer-

* Corresponding author.

tain circumstances can trigger programmed cell death (apoptosis) [15]. DSBs are considered to be particularly dangerous lesions because they are more difficult to repair than other types of DNA damage. For example, single-strand nicks are more easily repaired by any of the excision-repair pathways that involve using the undamaged anti-parallel strand as a template [16, 17]. In contrast, DSBs result in loss of integrity of both DNA strands. Furthermore, the biochemical configurations of the broken DNA ends are typically incompatible, and rejoining by a simple ligation step is not usually possible to repair the lesion [5].

There is comprehensive experimental evidence supporting a causal relationship between DSBs and genomic instability. Erroneous rejoining of DNA DSBs can induce deletion or insertion mutations and chromosomal translocations that are potentially carcinogenic [18–22]. Indeed, chromosomal translocations involving the Ig and T-cell-receptor loci are evident in many cancers of lymphoid origin, suggesting that they are the result of defective resolution of DSBs related to V(D)J recombination [10–13]. For example, in the B-cell malignancy, Burkitt's lymphoma, the *c-MYC* gene is often juxtaposed to the Ig heavy-chain genes as a result of incorrect chromosomal recombination [10–13]. More generally, the loss or amplification of chromosomal regions that is a feature of many cancer cells may be associated with inappropriate DSB repair. It has been suggested that erroneous DSB repair can lead to carcinogenesis in cases where the deleted genes are associated with tumor suppressor loci or amplified genes encode an oncogene [4, 22]. Moreover, and as discussed further below, defects in cellular responses to DSBs, including mutations in factors involved in signaling and repair, may increase cancer predisposition or could frequently be the initiating events of carcinogenesis [4, 22, 23].

Cellular responses to DSBs

It is accepted that the main cellular responses to DSBs include cell-cycle regulation, DSB repair, transcriptional and post-transcriptional activation of relevant genes (including those associated with repair), increases in cellular levels of deoxyribonucleotides and in certain cells induction of apoptosis [4, 22]. At least in some cases the overall DSB response is considered as a classical signal-transduction cascade [4, 22, 24]. These types of models imply that DSBs (signals) are detected by sensor proteins that activate protein kinase (transduction) cascades which result in amplification and diversification of the signal through a series of downstream effector molecules (response) (fig. 1).

Molecular mechanisms by which DSBs are detected have been proposed recently. It has been suggested that the checkpoint proteins Rad1, Rad9, Rad17 and Hus1 as well as other proteins are involved in recognition of DNA damage [25]. Findings in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* indicate that Rad1, Rad9, Rad17 and Hus1 or their homologs are essential for DNA damage-induced checkpoint activation and act in the initial phases of the DNA damage signaling pathway [26–29]. Importantly, it has been shown that Rad1, Rad9 and Hus1 display sequence (and possibly structural) homology with PCNA [30, 31]. PCNA is a sliding clamp

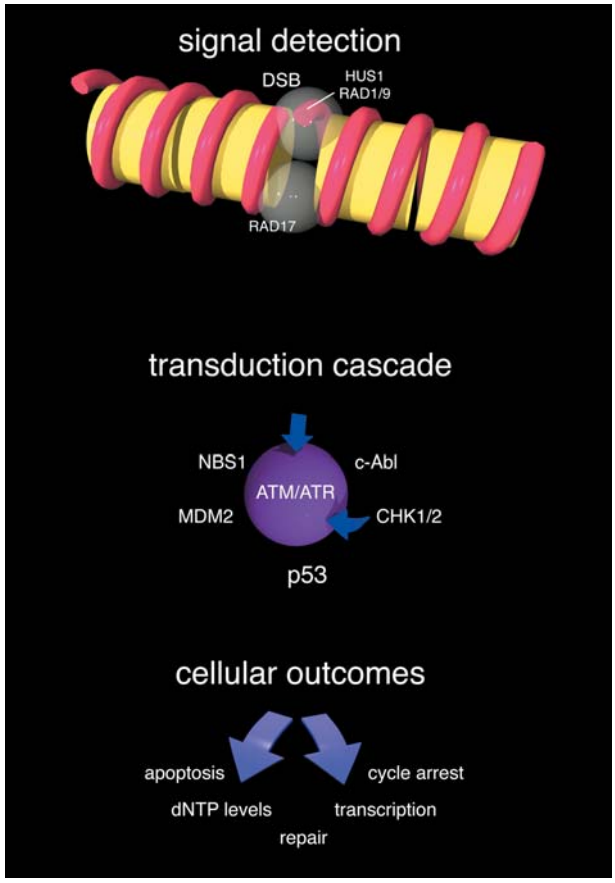

Figure 1. Schematic representation of cellular responses to DSBs. Sensor proteins detect the DNA damage and form nuclear foci in the vicinity of DSBs. The protein kinases ATM and ATR have central roles in the DSB damage-induced signaling pathway. Activation of ATM and ATR results in phosphorylation of downstream substrates that are involved in cell-cycle arrest, modulating transcription of stress response genes and cellular levels of deoxyribonucleotides (dNTP), DSB repair and apoptosis. In addition to the pathways discussed in the text, modification of CHK1/CHK2 and MDM2 by ATM/ATR is shown. Activated CHK1/CHK2 can effect cell-cycle arrest by phosphorylating p53 and/or by inhibiting Cdk2/Cyclin B1 activity. Modification of p53 by ATM and CHK1/CHK2 phosphorylation also results in inhibition of Cdk2/cyclin B1 activity (G_2 arrest) as well as inhibition of Cdk2/Cyclin E activity (G_1 arrest) [5]. The ATM and CHK1/CHK2 phosphorylations of p53 and/or modifications of MDM2 by ATM interfere with the binding of p53 with MDM2, which targets p53 for degradation [5].

protein that forms a homotrimeric toroidal structure around DNA and tethers DNA polymerase μ during replication [32, 33]. These homology analyses together with the findings that Rad1, Rad9 and Hus1 interact with one another in cell lysate and yeast two-hybrid experiments, have prompted the suggestion that Rad1, Rad9 and Hus1 form clamplike complexes that are involved in the detection of damaged DNA [25, 34–36]. The finding that Rad1, Rad9 and Hus1 form nuclear foci in response to DNA damage is consistent with this clamp-protein idea [37].

Clamplike structures require loading around the DNA by clamp proteins. It is well known, for example, that the clamp loader for PCNA is the heteropentameric structure replication factor C (RFC) [32, 33]. Classical RFC complexes recognize primer-template junctions and load the PCNA clamp around the DNA in an energy-dependent process [32, 33]. It follows that the clamp proteins Rad1, Rad9 and Hus1 also require a clamp loader to facilitate their function. Several lines of investigation suggest that the checkpoint protein Rad17 is involved in loading the Rad1-Rad9-Hus1 clamp around the DNA. Studies indicate that Rad17 exhibits structural homology with RFC and that the *S. cerevisiae* homolog of Rad17 (scRad24) interacts with four of the five RFC subunits [38–42]. Further analyses demonstrate that Rad17 interacts directly with Rad1 and provide evidence that Rad17 interacts with the Rad1-Rad9-Hus1 clamp in a similar way to the interaction between RFC and PCNA [25].

On the basis of the above observations, an accepted paradigm for the detection of DSBs is that a clamp loader consisting of Rad17 recognizes structural modifications induced by DNA damage [24, 25]. The Rad17 clamp loader then fixes the Rad1, Rad9, Hus1 clamp proteins around the DNA, converting them into extraction-resistant nuclear foci [25]. Recognition of DSBs by these sensor proteins may then result in the recruitment of DNA-processing proteins or in the activation of downstream DSB signaling targets.

The protein kinase, ataxia telangiectasia mutated protein (ATM), is a central component of the DSB signaling cascade in mammalian cells [4, 22, 43, 44]. A deficiency in ATM results in a predisposition to cancer and causes the neurodegenerative condition ataxia telangiectasia (A-T) [45]. Cells deficient in ATM are particularly sensitive to the effects of ionizing radiation and to radiomimetic agents [46, 47]. Moreover, A-T cells are defective in regulating ionizing radiation-induced G_1-S, S and G_2-M cell-cycle checkpoints [4, 48, 49]. The C-terminal region of ATM is homologous to the phosphatidylinositol 3-kinase-like kinases (PIKKs) [50]. The PIKK-related proteins have analogous kinase activities and phosphorylate target proteins on serine or threonine residues followed by glutamine [50, 51]. Following DSB-induced activation, ATM rapidly phosphorylates downstream substrates, including p53, MDM2, CHK1, CHK2, BRCA1 and NBS1, resulting in effects on cell-cycle regulation, DNA repair and apoptosis [4, 22, 52, 53]. In addition to DNA damage signaling, homologs of ATM in *S. cerevisiae* (Tel1p) and *S. pombe* (Tel1) are also involved in genomic surveillance and telomere metabolism [22].

It has been found that ATM substrates are phosphorylated in A-T cells, albeit with delayed kinetics [4, 54]. Phosphorylation of ATM targets in A-T cells is largely mediated by another PIKK family member, ataxia telangiectasia-related (ATR) protein [44, 54–56]. Homozygous inactivation of Atr results in inviable mouse or chicken B lymphocytes, and the $Atr^{-/-}$ genotype in mice causes early embryonic lethality [57, 58]. This phenotype is due to extensive chromosomal fragmentation, apoptosis and loss of clonogenicity [57, 58]. Furthermore, dominant-negative expression of ATR in mammalian cells results in sensitivity to a variety of DNA-damaging agents, including ionizing radiation, and to the DNA replication inhibitor hydroxyurea [59, 60]. These findings are consistent with a central role for ATR in DNA damage signaling and in recognition and repair of stalled DNA replication complexes [22]. Homologs of ATR in *S. cerevisiae* (MEC1p) and *S. pombe* (RAD3) are also key components in DNA damage surveillance and signaling pathways [61, 62].

As alluded to above, ATM and ATR phosphorylate some common substrates. The available evidence suggests that ATM and ATR function independently but in mainly overlapping pathways of DNA damage signaling. Despite the somewhat related functions, it is accepted that ATM mediates responses to DNA damage that is induced by ionizing radiation and radiomimetic drugs, whereas ATR is involved in responses to ultraviolet-induced DNA damage and other bulky lesions [59]. The details of DSB-induced ATM and ATR signaling pathways, particularly those relating to the effects on cell-cycle progression, have been reviewed extensively [4, 22, 43, 44, 63–65].

Briefly, cellular responses to DSBs other than cell-cycle regulation include, induction of apoptosis, activation of transcription of stress response genes, increases in cellular levels of deoxyribonucleotides and DSB repair. In cases where the extent of DNA damage is irreparable, certain cell types can enter programmed cell death [22, 66–69]. Although it is known that p53 signaling events represent an important component of apoptosis, the detailed processes that influence cell fate (survival or death) are not well understood [4, 22, 68]. Current thinking suggests that the decision whether to initiate reversible cell growth arrest or apoptosis is made early in the DNA damage signaling pathway, upstream of p53 [24, 70, 71]. Nevertheless, it is accepted that the intensity of DNA damage is the critical factor in directing the signaling cascade toward reversible arrest or apoptosis [24, 72]. Another cellular re-

sponse to DSBs is increase in transcription of stress response genes, which appears to be mediated by ATM phosphorylation of c-Abl, which in turn activates Jun kinases [63, 73]. Elevation of cellular levels of deoxyribonucleotides, which are essential for DNA synthesis processes during DSB repair, is a further aspect of the DNA damage response. This is considered to be of particular importance in non-dividing cells [22]. In mammalian cells, elevation of deoxyribonucleotide levels involves transcriptional induction of the ribonucleotide reductase p53R2 by p53, whereas budding yeast achieve a similar result by post-translational modification of the ribonucleotide reductase inhibitor Sml1p [72, 74]. Activation and recruitment of DSB repair factors is also a crucial component of the DSB-induced response cascade [4, 22].

DSB repair: non-homologous end-joining and homologous recombination

DSBs in mammalian cells are repaired by one of two distinct and complementary pathways – homologous recombination (HR) and non-homologous end-joining (NHEJ) – both of which have been reviewed recently [4, 5, 63, 75–79]. HR relies on extensive sequence homology between the recombining ends and essentially involves copying the missing information from an undamaged homologous chromosome [4, 22]. Thus, it is typically error-free and occurs without the loss of genetic information. This pathway is the one predominantly used by simple eukaryotes, such as the yeasts *S. cerevisiae* and *S. pombe*, to repair DSBs [80]. In contrast to HR, NHEJ does not require an undamaged DNA molecule and does not rely on extensive sequence homology. Instead, NHEJ involves processing of the broken DNA termini to make them compatible, followed by a ligation step [5]. In most cases, this pathway results in the loss of a few nucleotides at each broken end [5, 81, 82]. Hence, DSB repair by NHEJ is typically error-prone and is not a perfect process with respect to preserving genomic information. Similarly, a variation of HR known as single-strand annealing (SSA) leads to loss of genetic material [73]. SSA occurs when there are direct repeat sequences on each side of the damaged DNA ends. Following specific resection of both 5′ ends, the complementary sequences are annealed, leading to the loss of a repeat sequence and the intervening DNA [4, 73]. NHEJ is the more common DSB pathway in higher eukaryotes, and predominates in most stages of the cell cycle, particularly in G_0 and G_1 [4, 22, 5]. Furthermore, the NHEJ pathway is essential for repair of DSBs that occur during V(D)J recombination and Ig class-switch recombination in lymphocytes [5]. However, HR is also important for DSB repair in multicellular eukaryotes, particularly during the late S and G_2 phases of the cell cycle [4, 5, 22, 83].

NHEJ has been studied extensively in mammalian cells using genetic and biochemical techniques (fig. 2). It is proposed that the Ku70/Ku86 (Ku 86 is also called Ku80) heterodimer is the first protein to bind to broken DNA termini at the site of DSBs [5, 84–86]. Ku is abundant in cells and binds to DNA ends with high affinity in a non-sequence-dependent manner [84–86]. Binding of Ku to DNA recruits another PIKK family member, known as the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) [87–91]. It is noteworthy that in the absence of Ku, DNA-PKcs binds to DNA termini, albeit with about 100-fold lower affinity [89]. The binding of DNA-PKcs to DNA is a requirement for activation of its serine/threonine kinase activity [5, 87, 88]. Some studies suggest that phosphorylation of downstream target substrates by DNA-PKcs is an important component of the DNA damage signaling pathway; however, the precise contribution of DNA-PKcs is unclear [5, 89, 92]. Interestingly, autophosphorylation of DNA-PKcs results in dissociation of the DNA-Ku-DNA-PKcs complex [5, 92]. Following assembly of the DNA-Ku-DNA-PKcs complex and DNA end processing (discussed below), the DNA termini are ligated by the XRCC4-DNA-ligase-IV complex [5, 93–95]. The finding that the XRCC4-DNA-ligase-IV complex does not form a stable complex with DNA in the absence of the Ku complex has prompted the suggestion that Ku may recruit and load the ligase complex at the sites of DSBs [5, 96–98].

A somewhat controversial area of vertebrate NHEJ relates to the components involved in nucleolytic processing of

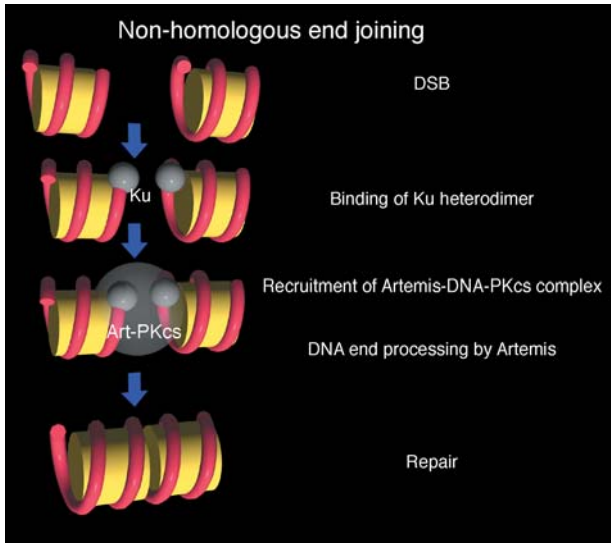

Figure 2. Schematic representation of non-homologous end joining. Following DSB induction, the heterodimer binds to the broken DNA termini. The Artemis-DNA-PKcs complex also binds to the DNA. Following DNA end processing, which involves nucleolytic cleavage of DNA by Artemis and possible gap filling by polymerases, the XRRC4-DNA ligase IV complex is recruited for the final ligation step.

broken DNA termini prior to the ligation step. It has been suggested that in mammals, the RAD50-MRE11-NBS1 complex, which possesses nuclease and helicase activities and localizes at nuclear foci that are associated with DSB, is involved in processing the broken DNA ends, both in NHEJ and HR [99–101]. It is known that NHEJ is affected in *S. cerevisiae* strains that are deficient in components of the analogous Rad50-Mre11-Xrs2 complex [102–104]. However, the genetic data available in mammalian cells raises questions regarding the importance of the RAD50-MRE11-NBS1 complex in NHEJ. It has been found that V(D)J recombination, which requires NHEJ for appropriate rejoining, is normal in Nijmen breakage syndrome, *NBS1*$^{-/-}$, cells and in cells that are homozygous for defective RAD50 alleles [105–107].

The recent eludication of the function of a protein known as Artemis has shed some light on the nucleolytic processes involved in NHEJ [5, 108–110]. Interestingly, the study of Artemis has allowed a unique connection between Greek mythology and science to be made. In quite intelligent ways the role of the Greek goddess Artemis in ancient mythology – as a guardian of women in childbirth (she was also guardian of wild animals and goddess of hunting) – has been related to the key role of the protein in the development of immune responses and in the DNA damage response pathway [5, 109]. A different approach in relaying information relates to the goddess' feminism and has involved revealing discussions of Artemis' cleavage patterns [109]. Nevertheless, it has been determined that Artemis has specific nuclease activity and that the protein is phosphorylated by and forms a complex with DNA-PKcs [5, 108]. In short, the Artemis-DNA-PKcs complex appears to be exclusively responsible for cleaving DNA hairpins in V(D)J recombination and is a critical component in resecting DNA termini (removal of 5′ and trimming of long 3′ overhangs) in NHEJ [5, 108]. Given the 5′ and 3′ overhang nuclease activity of the Artemis-DNA-PKcs complex, it has been argued that there may not be the need for another nuclease in NHEJ [5]. Furthermore, evidence that there may be no other nuclease involved in mammalian NHEJ is provided from the finding that Artemis-DNA-PKcs-null mammals are extremely sensitive to the effects of ionizing radiation [110, 111]. However, appropriate genetic knockouts will be required to be certain.

Another unresolved issue in NHEJ is related to the potential use of a polymerase in the process. One particular line of thought is that since the Artemis-DNA-PKcs complex could process the DNA until the two DNA ends are compatible, a polymerase for at least some NHEJ events may not be essential [5]. Nevertheless, it is known that the Pol4 protein is involved in filling in DNA gaps in *S. cerevisiae* end joining [5, 112]. The analogous homolog in multicellular eukaryotes has not been identified; however, there has been a report indicating that the Ku-DNA XRRC4-DNA ligase IV interacts with polymerase μ [113]. It is noteworthy that both Pol4 and pol-μ are members of the Pol-X polymerase family. Further studies will be required to identify which of the at least 15 mammalian polymerases are involved in NHEJ [5, 114].

Initially, the repair of DSBs by HR was characterized in bacteria and yeast. Genetic analysis of *S. cerevisiae* indicated that proteins coded by the RAD52 epistasis group of genes – Rad50, Rad51, Rad52, Rad54, Rad55, Rad 57, Rad59, Mre11 and Xrs2 – are important in HR [4, 22, 64]. In *S. cerevisiae*, an early event in HR is nucleolytic resection of the broken ends in the 5′ to 3′ direction [63]. This process involves the Rad50-Mre11-Xrs2 complex (NBS1, which is a direct substrate for ATM phosphorylation, is the mammalian homolog of Xrs2) [22, 115]. The resulting 3′ single-stranded DNA overhangs are then bound by Rad51 [63]. This process is facilitated by replication protein A (RPA), Rad54 and Rad52, which is known to bind to DNA termini [22, 116]. Notably, it has been suggested that in mammals Rad52 competes with Ku for binding to broken DNA ends [116]. This has led to the proposal that competition between Rad52 and Ku for binding DNA termini may be the critical event in determining whether NHEJ or HR is used to repair the DSB [116]. Nevertheless, following binding of Rad51 to the 3′ ends, the RPA-coated Rad51 nucleoprotein filament mediates a search for homologous sequences on an undamaged DNA duplex [22, 63]. Once a homologous region is located, Rad51 initiates strand exchange in which one of the damaged DNA strands invades the homologous duplex, forming a D-loop [22, 75]. This process is facilitated by Rad55 and Rad57 in yeast [63, 117]. The 3′ termini then prime new DNA synthesis, resulting in the extension of the damaged DNA by polymerases that copy information from the homologous undamaged partner. In meiotic cells, the DNA ends are ligated by DNA ligase I, and the Holliday junctions are resolved by nucleolytic cleavage [22, 63]. This process can result in either crossover or non-crossover gene conversion products, depending on the mode by which the Holliday junctions are resolved [75]. It has been suggested that mitotic recombination may not involve resolution of Holliday junctions, rather, it may coupled with replication [4, 118].

Studies have identified that mammalian homologs exist for all of the known gene products that are in involved in HR in *S. cerevisiae*, indicating that the basic HR pathway is conserved in higher eukaryotes [119–122]. However, it is accepted that the molecular mechanisms are more complex in higher organisms. For example, a number of RAD51 paralogs such as RAD51B, RAD51C and RAD51D and other proteins with some homology to the catalytic domain of RAD51, such as XRCC2 and XRCC3, have been identified in human cells [123]. It has been proposed that these facilitate the function of RAD51 in a similar manner to Rad55 and Rad57 in *S. cerevisiae*

[63]. Furthermore, mammalian cells possess additional proteins that are involved in HR that lack direct homologs in yeast. Two of the most important proteins in this regard appear to be the breast cancer susceptibility proteins BRAC1 and BRCA2.

It is clear that BRCA1 and BRCA2 are essential for efficient HR in mammalian cells. BRCA1 interacts with a wide range of cellular proteins and has multiple functions in DNA metabolism. Therefore, dissecting its role in HR has proved to be a difficult process. To date, the finding that, following ATM phosphorylation, BRCA1 and c-ABL phosphorylate RAD51 on several sites is probably the strongest evidence in support of a role for BRCA1 in HR [124–126]. In contrast a direct role for BRCA2 in HR has been established. BRAC2 interacts with RAD51 (RAD51 binds to a series of BRC motifs on BRCA2) and regulates the binding of RAD51 to DNA as well as the ability of the protein to form nucleoprotein filaments on DNA [127, 128]. Notably, the findings that BRCA2 interacts with histone acetylases and that BRCA1 is associated with the SWI/SNF chromosome-remodeling complex and interacts with histone acetylases suggest that a role of these proteins in HR may involve modulation of chromatin structure [129, 130].

DSBs and chromatin dynamics

The packaging of eukaryotic DNA with histones and accessory proteins into chromatin has important implications in DNA-processing events such as transcription, replication and repair [131–133]. The basic repeating unit of chromatin, the nucleosome, consists of ~160 bp of DNA wrapped around octamers of the core histones – two each of H2A, H2B, H3 and H4. Strings of nucleosomes are further compacted into 30-nm chromatin fibers via linker (H1) histones and looped domains [131, 134]. These are assembled into higher-order structures which are less well defined. Notably, it has been proposed that assembly of DNA into chromatin – even in the active con-

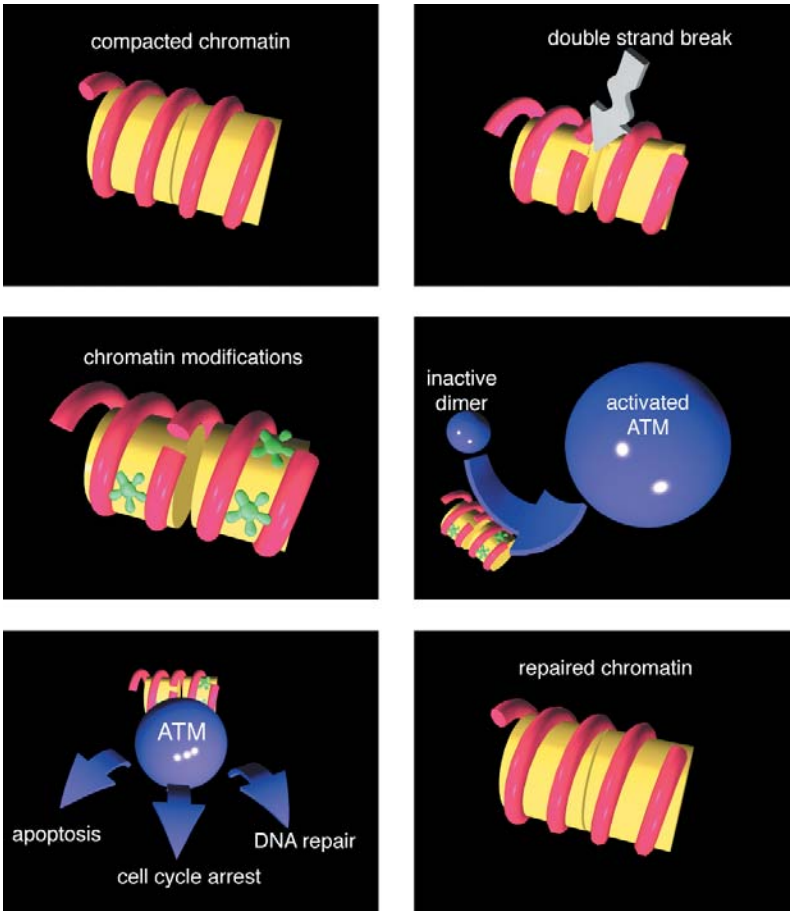

Figure 3. Proposed model for activation of ATM by DSB-induced chromatin remodeling. Following DSB induction, the core histones are modified (by phosphorylation and acetylation), resulting in the active chromatin conformation. The change in chromatin structure – from the compacted to the open conformation – triggers cross-phosphorylation of serine-1981 residues in ATM dimers. This results in the release of ATM monomers with active kinase domains which are critical components of the DSB response signaling pathway. The ATM monomers phosphorylate important downstream target substrates, resulting in effects on cell-cycle regulation, DNA repair and apoptosis.

formation (discussed below) – may be sufficient for maintaining the broken DNA termini in proximity following DSBs [5]. Also, it is known that this compaction limits the access of proteins to DNA and therefore affects all aspects of DNA processing [133–138]. For example, compaction of DNA into chromatin is accepted to be a principal mechanism by which transcription is repressed [135-138]. Similarly, the transcriptionally inactive (also referred to as closed or packed) chromatin conformation would prevent access of proteins involved in processing DSBs to genomic DNA. Importantly, nucleosomes are dynamic structures that can be modified to adopt a more open (also referred to as transcriptionally active or unpacked) conformation which facilitates the accessibility of proteins to DNA within chromatin [135–138]. Histone acetyltransferases which catalyze acetylation of the core histones (on the amino-terminal tails of lysine residues) and chromatin-remodeling complexes are important in modulating chromatin conformation [135–138].

It is known that chromatin modifications – such as phosphorylation of H2A and acetylation of H3 and H4 – in response to DSBs are important in altering chromatin conformation and allowing access of DNA damage-processing proteins to the DNA [139–142]. Furthermore, the findings of a recent landmark study provide exciting evidence that changes in chromatin conformation following DSBs may trigger widespread activation of the ATM kinase domain [143]. The model suggested by this study implies that a signal from modified chromatin results in cross-phosphorylation of serine-1981 residues in ATM dimers (fig. 3). Dissociation of inactive dimers results in the release of active ATM monomers which phosphorylate downstream substrates that are important in the DSB response pathway [143, 144]. Finally, another very recent study has revealed fascinating evidence that the linker histone H1.2 is involved in DSB-induced apoptosis [145]. It has been proposed that histone H1.2 transmits apoptotic signals from the nucleus following DSBs by translocating to the cytoplasm in a p53-dependent manner and promoting cytochrome c release from the mitochondria [145]. There is evidence to suggest that the Bcl-2 family protein, Bak, is involved in histone H1.2-induced cyochrome c release [145].

Concluding remarks

Although our understanding of the cellular responses to DSBs has increased greatly in recent years, there are still important gaps in our knowledge. Therefore, more research needs to be directed at characterizing the DSB response pathway in greater molecular detail. Regarding regulatory issues, it is very important to establish how the decision to undergo DSB-induced apoptosis or growth arrest is reached. Futhermore, it is important to determine how a DSB repair pathway is chosen, particularly in the S and G_2 phases of the cell cycle where both NHEJ and HR pathways are active. Indeed, it will be interesting to see the actual level of integration of the two pathways. The effects of chromatin remodeling and histone modifications on the DSB response pathway are also starting to come into light. Already, by considering the available data, it is tempting to suggest that DSB-induced chromatin modifications may represent the key triggering event of the DSB response pathway. It is expected that the cellular responses to DSBs in the context of chromatin will be explored more intensely in the next few years.

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