Early events in the mammalian response to DNA double-strand breaks

Lucy C. Riches, Anthony M. Lynch and Nigel J. Gooderham*

Department of Biomolecular Medicine, Faculty of Medicine, Imperial College London, Sir Alexander Fleming Building, London SW7 2AZ, UK
Department of Genetic Toxicology, GlaxoSmithKline, Park Road, Ware, Hertfordshire, SG12 0DP, UK

Physical and chemical agents that induce DNA double-strand breaks (DSBs) are among the most potent mutagens. The mammalian cell response to DSB comprises a highly co-ordinated, yet complex network of proteins that have been categorized as sensors, signal transducers, mediators and effectors of damage and repair. While this provides an accessible classification system, review of the literature indicates that many proteins satisfy the criteria of more than one category, pointing towards a series of highly co-operative pathways with overlapping function. In summary, the MRE11–NBS1–RAD50 complex is necessary for achieving optimal activation of ataxia–telangiectasia-mutated (ATM) kinase, which catalyses a phosphorylation-mediated signal transduction cascade. Among the subset of proteins phosphorylated by ATM are histone H2AX (H2AX), mediator of damage checkpoint protein 1, nibrin (NBS1), p53-binding protein 1 and breast cancer protein 1, all of which subsequently redistribute into DSB-containing sub-nuclear compartments. Post-translational modification of DSB responding proteins achieves a rapid and reversible change in protein behaviour and mediates damage-specific interactions, hence imparting a high degree of vigilance to the cell. This review highlights events fundamental in maintaining genetic integrity with emphasis on early stages of the DSB response.

Introduction

Double-strand breaks (DSBs) are the most biologically significant genotoxic lesions, increasing the likelihood of chromosome breakage and rearrangement, mutagenesis and loss of crucial genetic information. Cells actively replicating DNA are particularly vulnerable since unrepaird single-strand breaks (SSBs) may be converted to DSB upon collision with the progressing replication fork, a phenomenon observed to a higher degree in cells deficient in SSB repair (1). Mammalian cells respond to DSB by activating a multitude of proteins involved in signalling and repair pathways and, although the majority of lesions are efficiently repaired, the very nature of a single DSB poses such a threat to cell survival that DNA damage checkpoint proteins may be activated to send the cell into arrest. This provides time for repair to proceed or, in the case of overwhelming damage, apoptosis ensues.

Individuals unable to elicit a full DSB response are typically characterized by a radiosensitive phenotype and are predisposed to cancer, as exemplified by epidemiological studies involving ataxia–telangiectasia disorder [ataxia–telangiectasia-mutated protein (ATM) deficient] (2), ataxia–telangiectasia-like disorder (ATLID) (MRE11 deficient) (3) and Nijmegen breakage syndrome (NBS1 deficient) (4) -derived cell lines; patient cohorts and studies employing cells deficient in the DSB response have proved indispensable for investigating signalling and repair pathways.

Exogenous induction of DSB

DSB are directly generated by exogenous agents such as gamma irradiation (5) and the radiomimetic compounds bleomycin (6), neocarzinostatin (7) and calicheamycin, which induces a high ratio of DSB:SSB (1:3) (8). Specifically, irradiation exposure generates DNA fragments of size consistent with chromosomal damage rather than those produced during apoptosis, thus implying genotoxicity as a primary mode of action rather than the consequence of apoptosis-related fragmentation (9). Alternatively, compounds may exert a genotoxic effect via base modification or covalent binding to DNA, which generates DSB as a secondary lesion upon collision with the replication fork or as a by-product of repair. Among this type of damaging agent is the alkylating chemical methyl methane sulphonate (MMS).

MMS treatment results in the methylation of DNA bases and generates DSB in a time- and dose-dependent manner (10) upon collision of progressing replication forks with SSB intermediates of base excision repair (11,12). Consequently, the cell cycle is halted and DSB repair pathways are engaged, which involves the differential regulation of a subset of repair-associated genes (13,14). In contrast to direct base modification by MMS, the topoisomerase inhibitors induce DNA damage by covalent stabilization of chromatin-bound topoisomerases in the so-called stabilized cleavage complex (SCC). Physiologically, topoisomerases function to regulate DNA topography, e.g. by relaxing super-coiled DNA by nicking chromatin ahead of the progressing replication fork. In the case of topoisomerase I inhibitors, such as camptothecin, which introduce a single-strand nick, DSB may result upon collision of the SCC and active replication fork (15). Consequently, camptothecin toxicity is limited to replicating cells and so primarily elicits an ATM- and Rad3-related protein kinase (ATR) response (16), leading to the repair of lesions using homologous recombination. In contrast, the topoisomerase II inhibitor, etoposide, is a potent inducer of DSB (17) regardless of cell cycle stage, and although formation of a SCC is considered important to the mode of toxicity (18), other DSB-inducing mechanisms are postulated that lead to activation of the ATM response (19). Indeed, following etoposide treatment, a DSB...
response is implied by the observation of an elevated incidence of discrete phosphorylated H2AX (γH2AX) foci (20) and by the induction of DNA repair-associated genes (13,14,21). This is further supported by data obtained using in vitro and in vivo micronucleus assays (22,23). To summarize, although mechanistic details are unclear, it is understood that collapse of the replication fork may be necessary to generate DSB following exposure to camptothecin (1), while etoposide toxicity towards cancerous cells is directly attributable to the cytotoxicity of DSB, which are a potent trigger of apoptosis (9).

Sensing the Break . . . Early Events
The role of telomeric proteins during the DSB response
T-loops are a model developed to explain the phenomenon of telomeric end capping, which is hypothesized to prevent chromosome ends from being misinterpreted as damage signals. While conflicting data raise questions regarding the role of human telomeric proteins in the DSB response (24), telomeric repeat binding factor (TRF2) was reported to suppress ATM-dependent events (25,26) and the application of fluorescent-tagged proteins has revealed transient TRF2–DSB interactions within seconds of irradiation (26,27). Although the importance of TRF2 in the DSB response is unclear, TRF2 absence negatively impacts homologous recombination repair, by the failure of RAD51 to accumulate into discrete, DSB-related repair centres (28). It is plausible to speculate that TRF2 not only contributes to the restructuring of damaged chromatin but also mediates strand invasion, in order to promote accurate recombination between the damaged and intact sister chromatids (28).

A role for helicases during the DSB response
Bloom syndrome protein (BLM) migrates to γH2AX-marked chromatin, in an ATM-independent manner, with rapid kinetics (i.e. within 60 sec of DSB induction) (29,30). In the vicinity of irradiation-induced breaks, BLM interacts with the homologous recombination repair protein RAD51, and replication protein A (RPA) also, which coats regions of single-strand DNA (ssDNA) (31). These interactions, which are typical of replication fork-associated stress, are limited to late S/G2-phase cells (31), thus implicating BLM as a possible modulator of homologous recombination (N.B. homologous recombination primarily proceeds in late S/G2-phase cells due to the presence of an intact sister chromatid to provide a template for repair). In support of this notion, BLM is also described to aid the recruitment of phosphorylated p53 to replication-associated breaks, with a predominant role in the response to stalled forks (32) and possibly influencing the mode of repair (33). Analogous to Werner’s syndrome protein, which displays an affinity for DSB per se (34), BLM possesses helicase activity, a function thought to be necessary for achieving optimal ATM activity towards substrates, possibly by modulating chromatin structure to generate a common damage signal or by enhancing chromatin accessibility to mediator and effector proteins (30). It should be noted that it is not yet clear from review of the literature whether BLM protein is important in non-replication-associated DSB.

Multiple roles for the MRE11–NBS1–RAD50 complex during the DSB response
The MRE11–NBS1–RAD50 (MRN) complex comprises a heterotetramer of MRE11 and RAD50, which is associated with NBS1, and contributes to telomere maintenance (35–37) and surveillance of the progressing replication fork (38,39) in addition to its role in DSB signalling (40,41). In terms of the mammalian DSB response, the MRN complex is of primary importance for signalling breaks and promoting lesion repair, which is largely attributable to the following characteristics:

- Activation of ATM catalytic function (via interaction with NBS1);
- Retention of ATM at DSB to achieve signal propagation;
- Nucleolytic processing of DSB to generate an intermediate signal for ATR and
- Tethering of sister chromatids prior to homologous recombination.

Activation of ATM and signal propagation
The MRN complex is of structural and functional importance during the early DSB response, by tethering DNA ends and recruiting ATM, thereby generating signalling complexes comprising damaged DNA and catalytically active ATM (41,42). NBS1, which contains an MRE11 interaction domain within its carboxyl terminus, is essential to cell viability by mediating nuclear localization of the MRN complex (41,43–46) and also through its ability to interact with ATM via a carboxyl terminus-encoded motif (41). Specifically, NBS1 is necessary for promoting a subset of downstream ATM-dependent events including the intra-S-phase checkpoint via structural maintenance of chromosome 1 phosphorylation and apoptosis by the proapoptotic protein BH3 domin-only protein (47–49). MRE11 is also crucial for optimal ATM signalling, and for cells to engage in appropriate cell cycle checkpoints, particularly following low levels of DSB damage (50). The fundamental role of the MRN complex may be inferred from several experimental observations: (i) impaired formation of signalling complexes in cells from ATLD (MRE11 deficient) patients (42), (ii) failure of viable offspring in NBS1-deficient mice (51), (iii) attenuated ATM signal transduction following knockdown of NBS1 expression in human cells (52) and (iv) diminished MRN levels in neoplastic breast cells (53). Consequently, both NBS1 and MRE11 are necessary for optimal ATM activity in mammalian cells.

Following MRE11 binding to DNA, discrete MRN complexes situated on adjacent DNA-free ends associate via RAD50 interactions to connect DNA ends prior to repair (54–56). RAD50 belongs to the structural maintenance of chromosome protein family (56) and exhibits both ATP-binding and hydrolysis activity (57), which is crucial for RAD50 dimerization (58,59) and hence stabilization of the DSB by the MRN complex. Adenylate kinase activity displayed by RAD50 is thought to catalyze the tethering of DNA molecules (60), thereby holding broken DNA in close proximity to ensure correct repair.

Nucleolytic processing of DSB to generate an intermediate signal for ATR
As a macromolecular complex, the MRN proteins act in concert to partially unwind the DNA duplex and resect DNA ends (3’) (57), with DNA end processing activity being attributable to the exo- and endonuclease activity of MRE11 (56). Most likely, DNA end processing generates a universally recognized marker of damage from the multitude of lesions
encountered, from directly induced DSB to those comprising chemical bound ends (i.e. following covalent binding of chemical to DNA).

The MRN complex during replication stress

Under physiological conditions, the MRN complex is found in association with chromatin during DNA replication, most likely so as to initiate a rapid response to replication fork-related stress (39,61). The MRN complex is implicated in functional aspects of homologous recombination (62) in an ATM-independent manner (63), consistent with the notion of homologous recombination being the predominant repair pathway for replication-associated DSB. In accordance with this, sub-nuclear microcompartments containing replication fork-associated stress proteins ATR–ATRIP, a subset of the homologous recombination-related RAD proteins, breast cancer protein (BRCA) 1 and 2, RPA and the MRN complex are observed in association with replication forks in unstressed cells (64).

Transient MRN–chromatin contacts are reinforced through DSB-induced phospho-dependent interactions, postulated to involve the forkhead-associated (FHA) and breast cancer carboxyl terminus (BRCT) functional domain encoded within the N-terminus of NBS1 and γH2AX (43,62,65,66). Within γH2AX compartments, the MRN complex also contacts BRCA1 (67) and mediator of damage checkpoint protein 1 (MDC1) (68), the latter acting to reinforce the transient nature of initial MRN contacts (64).

The role of NBS1 in responding to DSB

Functional analyses of NBS1 have revealed that the amino terminus-encoded FHA and BRCT domains are crucial for accumulation of the MRN complex at DSB (43–46) and that ATM/ATR consensus phosphorylation sites within the central region of NBS1 may promote more stable interactions at a DSB (45). As mentioned above, the NBS1–ATM interaction is important for achieving an optimal ATM response (see Figure 1A) (41,69), which is supported by observations that events downstream of ATM (i.e. CHK2 phosphorylation) appear attenuated following the inhibition of NBS1 expression in human cells, using small interfering RNA (52). Furthermore, cells deficient in NBS1 or expressing various truncated forms of the protein are typically radiosensitive, characterized by diminished phosphorylation of ATM substrates and intra-S and G2/M cell cycle defects (41,49,70,71). Intracellular indications have been supported using cell-free systems and mouse models, albeit with diminished dependence on MRN-mediated activation of ATM, concomitant with elevated DSB (40,42,51), although it is unclear whether this is the consequence of fundamental species differences.

In replicating cells, the ATR pathway predominates, and the role of NBS1 in mediating MRE11 nuclear localization is of greater significance than the influence exerted over ATM activation. This most likely reflects the ability of NBS1 to achieve nuclear localization of MRE11/RAD50, which proceeds to stabilize DSB lesions by tethering sister chromatids prior to recombination repair (42,62). Indeed, the essential nature of NBS1 is largely attributable to the contribution of the intact, nuclear MRN complex to maintaining genome stability, but also through a postulated role of NBS1 in telomere maintenance (37).

NBS1 is distinct from several counterpart proteins, in that both γH2AX-dependent (70) and -independent associations are observed, the latter more prominent in replicating cells and involving regions of MRN-processed ssDNA (64). Crucially, chromatin immunoprecipitation analysis reveals that NBS1 (in the context of the MRN complex) directly associates with the break, whereas γH2AX is situated adjacently (69).
Transducing the DSB response

The majority of DSB responding proteins become activated by addition of a phosphate catalyzed by the phosphoinositide 3-kinase (PI3-K)-like kinase proteins ATM, ATR and DNA-dependent protein kinase catalytic subunit protein (DNA-PKcs). Consistent with widely observed post-translational modification, protein abundance is seldom altered in response to DSB. Rather, phosphorylation achieves a rapid and reversible response to DSB, hence imparting a high degree of vigilance to the cell.

Interaction of ATM, ATR and DNA-PKcs with partner proteins NBS1, ATRIP and Ku80, respectively (see Figure 1A), is mediated through highly conserved carboxyl-terminal motifs, an association which is considered indispensable for optimal kinase activity (72).

Although the PI3-K-related kinases exhibit broad substrate specificity and mediate phosphate transfer to common residues of H2AX and P53-binding protein 1 (53BP1) (73,74), they become activated upon distinct stimuli. It is thought that ATM autophosphorylation is a sensitive and specific marker of DSB (75), and experimental data suggest a threshold of 19 DSB per cell, necessary for fully activating downstream events (76). In contrast, the specific trigger for ATR–ATRIP appears to be ssDNA exposed during the processing of UV-light-induced replication-associated stress (i.e. at stalled forks) (39) or by ssDNA exposed during the processing of UV-light-induced thymidine dimers (78). Despite ATM and ATR activity being largely coupled to distinct branches of the DNA damage response and activated upon discrete stimulus, recent experimental data have implied a degree of pathway cross-talk (79).

Specifically, both kinases are activated following irradiation, although the role of ATR appears limited to late S/G2-phase (79). ATM proceeds, resulting in dissociation into highly mobile monomers (see Figure 1B) (88), of which a fraction becomes phosphorylated following DSB induction, achieving a plateau within 30 min, and visualization of antibody-labelled foci reveals an increase in size and number over time, which varies with the radiation source and dose, thus confirming the amplification of signal through the surrounding chromatin, analysis of focus size and number has proved an accurate indication of dose-related DSB in many studies (10,20,21,97,99,100). Detailed studies of foci attributes have detailed studies of foci attributes have revealed an increase in size and number over time, which varies with the radiation source and dose, thus confirming the evidence to support ATR-dependent activation of ATM in response to inhibitors of replication, UV light (73) and hyperoxia (83). A third member of the PI3 kinases, DNA-PKcs, exhibits a degree of functional redundancy in the phosphorylation of H2AX in NBS1- and ATM-deficient cell lines (72,84).

ATM activation

ATM exists in the nucleus primarily as dimers (85) in association with protein phosphatase 2A (PP2A), which seemingly prevents accumulation of trans-phosphorylated, kinase-active ATM under physiological conditions (86). Specifically, upon DSB stimulus (75), the ATM–PP2A interaction is abolished (87), and trans-phosphorylation of ATM proceeds, resulting in dissociation into highly mobile monomers (see Figure 1B) (88), of which a fraction becomes chromatin associated (85,89). Mutagenesis studies confirm that ATM activation is mediated by autophosphorylation at serine-139 by PI3-K, mammalian chromatin, and in response to DSB, protruding tails become rapidly phosphorylated at serine-139 by PI3-K like kinases, including ATM, ATR and DNA-PKcs (84,97). Whereas ATM and DNA-PKcs display functional redundancy in phosphorylating H2AX following irradiation-induced DSB (84), ATR appears more important for replication fork-associated damage (66) and those arising following camptothecin exposure (66).

H2AX: A Universal Biomarker of DSB

Histone 2AX phosphorylation

H2AX is a relatively rare histone distributed throughout the mammalian chromatin, and in response to DSB, protruding tails become rapidly phosphorylated at serine-139 by PI3-K like kinases, including ATM, ATR and DNA-PKcs (84,97). Whereas ATM and DNA-PKcs display functional redundancy in phosphorylating H2AX following irradiation-induced DSB (84), ATR appears more important for replication fork-associated damage (66) and those arising following camptothecin exposure (66).

H2AX is among the earliest of substrates to be phosphorylated following DSB induction, achieving a plateau within 30 min, and visualization of antibody-labelled foci indicates that γH2AX covers up to 2 Mbp of chromatin per DSB, thus providing a useful marker of damage (98). Despite the amplification of signal through the surrounding chromatin, analysis of focus size and number has proved an accurate indication of dose-related DSB in many studies (10,20,21,97,99,100). Detailed studies of foci attributes have revealed an increase in size and number over time, which varies with the radiation source and dose, thus confirming the...
value of γH2AX analysis (101). Importantly, in contrast to early reports, current data imply γH2AX formation as the direct consequence of DNA-free ends, as opposed to epigenetic alterations, such as chromatin relaxation (10,102).

Although H2AX phosphorylation appears to be a universal marker of genotoxic insult, experimental evidence suggests that γH2AX is not absolutely necessary to instigate the ATM signal transduction pathway (92,103). Rather than being a prerequisite for repair, γH2AX behaves as a platform onto which DSB responding proteins are concentrated to amplify the initial signal (103). Resultant foci of DSB responding proteins are commonly referred to as irradiation-induced foci, having been first identified following exposure to irradiation (67,104), although chemical exposure has been shown to induce such foci also. The importance of phosphopeptide recognition motifs in mediating protein interactions during DSB signalling and repair is widely described (43,46). It is of particular interest to note that a recent report suggests the post-translational modification of alternative histones, which likely contribute to the cells decision to commit to DSB repair via homologous recombination or non-homologous end joining (NHEJ) (105). Thus, it is feasible to postulate that alternative histone modifications and repair processes compensate for the absence of H2AX. Events leading to H2AX phosphorylation with subsequent accumulation of key DSB proteins are depicted in Figure 1B.

Despite the strong correlation of γH2AX foci with DSB-inducing treatment, it is important to note that H2AX also becomes phosphorylated during early apoptosis, most likely reflecting the excess of free DNA ends generated by genome laddering (106,107).

**Phosphopeptide-binding domains**

Clusters of serine and threonine residues are often found in readily accessible regions for rapid phosphorylation (108), which mediates phosphopeptide-specific associations between proteins to encourage their accumulation into DSB-containing microcompartments.

Two major phosphopeptide recognition domains contribute to the accumulation of proteins into discrete nuclear foci, the BRCT and FHA domain (46,68,70,109–113). The pivotal role of structural motifs is highlighted by the multitude of DNA damage-associated BRCT-containing proteins and observations that BRCT mutations or ectopically expressed BRCT peptides significantly diminishes focus formation (68,70,109,111,114). Although of limited sequence homology, BRCT repeats exhibit significant structural identity (111,113).

The phosphopeptide binding of proteins appears to display differential preference for the target encompassing sequence, which may explain protein-specific interactions (109). Proteins BRCA1, 53BP1, MDC1, NBS1, PTIP, Apraxin and poly-nucleotide kinase-like factor (PNK-like factor) and TOPB1, all contribute to DSB repair, or cell cycle signalling, and recognize short, specific motifs, of which phosphorylation is central for mediating association (110,115,116).

**Phosphopeptide recognition domains in DSB relevant proteins**

**The MDC1**

MDC1 (117) is hyperphosphorylated in response to DSB (118) and becomes stably associated with γH2AX and phosphorylated 53BP1, through dual C-terminal BRCT repeats (109,113,119), with rapid kinetics, reaching steady-state levels within 10–15 min of insult (113,117,120). In contrast with several DSB responding proteins, MDC1 accumulates at DSB regardless of cell cycle (64,93), and association with proteins involved in all aspects of the response from signalling (NBS1) through repair (i.e. RAD51 during homologous recombination) (121) indicates a central role for MDC1. Parallels may be drawn with NBS1 function, such that both proteins interact with ATM through FHA domains (69,93) and that cell cycle checkpoint-defective phenotypes are observed in NBS1- or MDC1 (i.e. CHK1- and CHK2-dependent intra-S and G2/M-phase checkpoints, depending on whether damage is signalled by ATR (CHK1) or ATM (CHK2))-deficient cells (41,93,117). Although MDC1 and NBS1 follow similar migration kinetics to DSB, MDC1 apparently participates in more stable interactions at the site of damage (96,120).

A growing body of data indicates the association of MDC1, with the phosphorylated forms of ATM, BRCA1, RAD51 and members of the intact MRN complex via FHA phosphopeptide recognition domain interactions (64,68,93,118,119,121), and while BRCA1 and NBS1 contact chromatin independently of MDC1, prolonged retention at DSB is abolished in MDC1-/- models (64). Over-expression of the FHA motif interferes with MRE11 and MDC1 accumulation at sites of DSB, thus placing importance on this domain for mediating protein recognition during the DSB response (118,119). It is unclear whether early responding proteins are displaced from MDC1 associations by proteins arriving at the DSB at later time points, although observations that only a fraction of MDC1 binds RAD51 (121) favour the interaction of proteins in discrete MDC1-containing foci. Interestingly, MDC1 co-immunoprecipitates with members of the MRN complex and ATM and also interacts with RAD51 in unstressed cells, raising the possibility that a subset of DSB responding proteins are recruited to γH2AX as part of a multiprotein genome surveillance complex (117,121).

**The 53BP1**

53BP1 is hyperphosphorylated by ATM and ATR (74) and accumulates into discrete foci following DSB in association with γH2AX, MDC1, ATM and at later time points with repair proteins such as RAD51 and RAD51C in the case of homologous recombination proficient late-S/G2 cells, the dispersal of which coincides with completion of repair (64,103,119,122). Evidence suggests that initial chromatin contacts are mediated through the DNA-binding Tudor domains of 53BP1 (123), although γH2AX association is necessary to facilitate 53BP1 retention (124), optimal phosphorylation of certain downstream ATM substrates (112) including BRCA1 (125) and to elicit an appropriate G2/M checkpoint (103).

**The BRCA1**

Cells defective in BRCA1 exhibit genome instability, diminished homologous recombination (126) owing to impaired RAD51 regulation and a preference for irradiation-induced repair using the error-prone pathways (127,128). Multiple phosphorylation motifs are described, hypothetically permitting BRCA1 to elicit differential responses to ATM and/or ATR stimuli (129,130). Regardless of DSB stimulus, BRCA1 redistributes into distinct microcompartments, along with γH2AX, MDC1 and the MRN complex (specifically RAD50) ATR and at later times repair proteins such as RAD51 (64,67,93,113,127,129).
Under the control of ATM and CHK2, BRCA1 influences the fidelity of DSB repair by NHEJ (128), and in a parallel pathway, BRCA1 disruption results in a decreased number of DSB processed by homology-directed repair (127,131). Specifically, retention of BRCA1 at DSB through phospho-specific interactions with MDC1 appears to encourage BRCA1 interaction with members of alternative repair pathways and thus provides a switch between repair mechanisms (64,93,132). In a similar manner to NBS1, BRCA1 becomes compartmentalized into ssDNA-containing chromatin regions, indicative of replication stress, and also redistributes into γH2AX/MDC1/53BP1-associated foci, the latter more widely implicated in irradiation-induced DSB (64). Thus, BRCA1 is implied as a universal indicator of DSB and co-ordinator of repair processes in mammalian cells.

Resolving the break
Typically, the repair of DSB proceeds by processing and ligation DNA ends using NHEJ, which is potentially error prone, or by employing homologous recombination, which restores the original DNA sequence. Research in this field indicates a strong dependence on cell cycle stage, with quiescent cells favouring NHEJ, while replicating and mitotic cells, which contain a homologous sister chromatid that provides a template for recombination repair, preferentially engage in homologous recombination (122,133,134). Thus, homologous recombination is pertinent to the repair of DSB generated during replication and as such is anticipated to be of greater consequence for repairing topoisomerase inhibitor-related damage. In the event of overt damage, cells may be eliminated via apoptosis, which is promoted by various DSB responding proteins including p53 and NBS1 (48,135). A full description of the role of homologous recombination and NHEJ in resolving DNA DSB is beyond the scope of this current review and reader referred to previous reviews (110,111).

Summary
Following exposure to DSB-inducing agents, mammalian cells elicit a multifaceted response comprising the activation of cell cycle checkpoints and initiation of repair or apoptosis pathways as appropriate. Among the earliest responding proteins are the PI3 kinases: ATM, ATR and DNA-PKcs. While DSB act as a direct trigger for ATM, ATR is more specifically activated by regions of RPA-coated ssDNA, exposed at stalled replication forks and also following nucleolytic resection (by the MRE11 component of the MRN complex) of irradiation-induced DSB during late-S/G2 phase. In contrast, DNA-PKcs appears to functionally compensate for both ATM and ATR activity to a limited extent. Thus, depending on the initial stimulus and cell cycle stage, ATM can activate the ATR pathway as a downstream event and the reverse situation has been reported also, so demonstrating a high degree of cross-talk between related pathways. Activated kinases are rapidly recruited to the damaged chromatin, where H2AX becomes phosphorylated, which participates in associations with mediator proteins such as MDC1 and NBS1, which in turn provide a scaffold upon which further DSB responding proteins are recruited. It is believed that this retains kinase substrates in DSB-containing sub-nuclear compartments along with catalytically active kinases to promote substrate phosphorylation and subsequently elicit an appropriate cellular response. Although proteins such as MDC1 and NBS1 have been widely described as mediator proteins that function in signal propagation, it is clear from review of the literature that they also participate in the early stages of the DSB response, by achieving optimal ATM activation, thus act both up- and downstream of ATM. Importantly, the MRN complex is recognized as an integral player in the DSB response, by converting free ends into a signal recognized by ATR, tethering sister chromatids prior to homologous recombination and amplifying the ATM response.

Funding
Biotechnology and Biological Sciences Research Council studentship (to L.C.R.); GlaxoSmithKline.

Acknowledgements
Conflict of interest statement: None declared.

References


L. C. Riches et al.

N- and C-terminus of NBS1 and is dissociated with ATM functions. Oncogene, 26, 6002–6009.


Mammalian response to DNA double-strand breaks


Received on February 19, 2008; revised on June 24, 2008; accepted on June 24, 2008