Review

ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response

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In vertebrate cells, the DNA damage response is controlled by three related kinases: ATM, ATR, and DNA-PK. It has been 20 years since the cloning of ATR, the last of the three to be identified. During this time, our understanding of how these kinases regulate DNA repair and associated events has grown profoundly, although major questions remain unanswered. Here, we provide a historical perspective of their discovery and discuss their established functions in sensing and responding to genotoxic stress. We also highlight what is known regarding their structural similarities and common mechanisms of regulation, as well as emerging non-canonical roles and how our knowledge of ATM, ATR, and DNA-PK is being translated to benefit human health.

Introduction

DNA in cells is continually being damaged in many ways. These range from DNA-base mismatches introduced during DNA replication, other base adducts and cross-links generated by chemicals, ultraviolet light (UV)-induced base damage, plus single- and double-strand breaks produced by abortive topoisomerase actions, chemical reactions, or ionizing radiation (IR). To pass their genomes on to the next generation intact, all organisms have evolved DNA repair pathways plus associated processes to respond to such damage and, in most instances, repair it faithfully (Ciccia and Elledge, 2010; Jackson and Bartek, 2009). In general, a cell's DNA damage response (DDR) involves DNA lesion recognition, followed by initiation of a cellular signaling cascade to promote DNA repair, which can be aided by a pause in cell-cycle progression (checkpoint activation).

In concert with such events, cells mediate other responses, including modulation of chromatin structure and transcription, both at sites of DNA damage and more globally. Notably, many DNA lesions become particularly toxic when encountered by the DNA replication apparatus, necessitating additional DDR mechanisms to deal with such lesions and allow accurate genome duplication. In multi-cellular organisms, certain cells with extensive DNA damage permanently exit the cell cycle (senescence) or undergo programmed cell death (apoptosis), presumably to mitigate the propagation of potentially mutated cells leading to cancer or other age-related pathologies. DNA lesions also serve as intermediates in certain biological processes, such as DNA demethylation, meiotic recombination, and generation of antibody diversity in the immune system (Alt et al., 2013; Jackson and Bartek, 2009).

Like many intra-cellular signaling cascades, DNA damage signaling is driven by protein phosphorylation. All eukaryotic genomes encode at least one of the three members of a family of phosphoinositide 3-kinase (PI3K)-related kinases (PIKKs; Abraham, 1998) with principal roles in activating the DDR. In this review, we provide a historical perspective on the identification of the three DDR PIKKs and discuss our current understanding of their functions, mechanisms of activation and regulation, structural similarities, and the consequences of their deficiencies. We end by discussing how understanding these kinases is relevant to human health and disease.

A Historical Perspective
Discovery of DNA-PK

In 1985, it was discovered by accident that addition of double-stranded DNA (dsDNA) to animal cell extracts increased the phosphorylation of certain proteins (Walker et al., 1985). This finding was serendipitous because the RNA preparations being used to study protein synthesis were contaminated with dsDNA. Fortunately, these seminal experiments provided evidence for the existence of a kinase that could be activated specifically by dsDNA. This DNA-dependent protein kinase (DNA-PK) activity was later partially purified and reported by three independent groups (Carter et al., 1990; Jackson et al., 1990; Lees-Miller et al., 1990). Kinase activity was associated with a particularly large polypeptide of >300 kDa (Carter et al., 1990; Lees-Miller et al., 1990), later named the DNA-PK catalytic subunit (DNA-PKcs; Blunt et al., 1995). Subsequent work established that effective DNA-PKcs recruitment and activation by DNA require Ku, a heterodimer comprising two subunits of ∼70 and 80 kDa (Dvir et al., 1992; Gottlieb and Jackson, 1993). Ku was originally identified as a nuclear factor in human cells reacting with sera from autoimmune disorder patients (Mimori et al., 1981) and so-called after the first two letters of the name of the patient whose serum was used to identify it. Notably, Ku had been shown to preferentially bind dsDNA ends (Mimori and Hardin, 1986), which, together with the observation that DNA-PK was activated by linear but not supercoiled plasmid dsDNA in vitro (Jackson et al., 1990), led to the realization that DNA double-strand breaks (DSBs) might also activate DNA-PK in vivo (Gottlieb and Jackson, 1993).

Prior to this, a set of radiosensitive, DSB-repair-defective rodent cell lines had been isolated and assigned to various complementation groups, but causative genetic defects had not been identified (Jeggo et al., 1995). Promoted by the potential role for DNA-PK in DSB repair, several groups established that Ku, DNA-PKcs, and their associated activities were defective in several such cell lines (Blunt et al., 1995; Finnie et al., 1995; Getts and Stamato, 1994; Peterson et al., 1995; Rathmell and Chu, 1994; Smider et al., 1994; Taccioli et al., 1994). Shortly after, DNA-PKcs was found to be absent in a radiosensitive human glioma cell line (Lees-Miller et al., 1995) and mutated in the radiosensitive, severe combined immunodeficient (SCID) mouse identified several years earlier (Blunt et al., 1995; Bosma et al., 1983; Kirchgessner et al., 1995). Taken together, these observations established that DNA-PK was crucial for DSB repair, by a pathway now known as non-homologous end joining (NHEJ).

Although the genes encoding the Ku70 and Ku80 subunits of DNA-PK had been cloned at this stage (Reeves and Sthoeger, 1989; Yaneva et al., 1989), the *PRKDC* gene encoding DNA-PKcs had not. A breakthrough came in early 1995, when the DNA-PKcs cDNA was cloned and found to encode a colossal ~460-kDa polypeptide (Hartley et al., 1995). Notably, the DNA-PKcs kinase domain was found to be most similar to that of the phospholipid kinase PI3K, rather than a canonical protein kinase. This was surprising, as DNA-PK displayed no detectable activity toward lipids (Hartley et al., 1995). DNA-PKcs also appeared strikingly similar to the product of a recently cloned gene mutated in human patients with a disease called ataxia-telangiectasia (A-T) (Savitsky et al., 1995).

Discovery of ATM

A-T is a rare inherited autosomal-recessive genetic condition (Taylor et al., 2015), characterized by dilated blood vessels (telangiectasia) and progressive neurological decline, resulting in a lack of voluntary movement coordination, including gait abnormality (ataxia). A-T patients also display immunodeficiency and predisposition to malignancies, especially lymphoid tumors. After some A-T patients developed severe complications following cancer radiotherapy (Gotoff et al., 1967), it was found that cells from A-T patients are profoundly radiosensitive (Taylor et al., 1975). This radiosensitivity was also apparent at the chromosomal level, suggesting a connection between the gene product mutated in A-T and DNA repair (Taylor et al., 1976). An important subsequent observation was that, while replicative DNA synthesis is markedly inhibited when normal cells are exposed to IR, this does not occur in A-T cells, which instead display radio-resistant DNA synthesis (Houldsworth and Lavin, 1980; Painter and Young, 1980). Later work showed that A-T cells are defective in establishing both the G1/S and G2/M cell-cycle checkpoints in response to IR (Imray and Kidson, 1983; Nagasawa and Little, 1983; Zampetti-Bosseler and Scott, 1981). A major insight into why the G1/S checkpoint is defective in A-T cells came when it was found that the tumor suppressor protein p53 is not induced properly in these cells after IR (Kastan et al., 1992).

When the A-T mutated (ATM) gene was identified (Savitsky et al., 1995), it was found that the C terminus of the predicted ATM protein contained a PI3K-like kinase domain. Although this initially suggested that ATM might function in phospholipid

signaling, we now know that it, like DNA-PKcs, is a protein kinase. Compared to proteins known at the time, ATM was found to be most homologous to Tel1, a budding yeast protein involved in controlling telomere length (Lustig and Petes, 1986) and now considered to be the yeast ATM ortholog (Greenwell et al., 1995; Morrow et al., 1995). Significantly, ATM was also found to share homology with two other yeast proteins implicated in DNA repair and cell-cycle checkpoint control: budding yeast Mec1/Esr1/Sad3 and fission yeast Rad3 (Savitsky et al., 1995).

Discovery of ATR

Mitosis entry checkpoint 1 (*MEC1*) is an essential budding yeast gene originally identified in a genetic screen for S and G2 checkpoint defects (Weinert et al., 1994). It was independently retrieved in genetic screens for hypersensitivity to the replication inhibitor hydroxyurea (HU) and the alkylating agent methyl methanesulfonate and was designated as *SAD3* or *ESR1*, respectively (Allen et al., 1994; Kato and Ogawa, 1994). The gene was cloned in the latter study and a C-terminal PI3K-like kinase domain identified. Sequence similarity between Mec1/Esr1/Sad3 (hereafter Mec1) and fission yeast Rad3 was also recognized, this being noteworthy because *rad3* mutants were known to be hypersensitive to UV, IR, and HU and to display checkpoint defects (al-Khodairy and Carr, 1992; Jimenez et al., 1992; Nasim and Smith, 1975; Rowley et al., 1992).

While the *RAD3* gene was sequenced shortly afterward (Seaton et al., 1992), the sequence was incomplete and the C-terminal kinase domain was not initially identified. Only when the similarity of Rad3 to the newly identified Mec1 and ATM proteins was noted, was the Rad3 locus reanalyzed, its full-length nature determined, and its PIKK domain identified (Bentley et al., 1996). The latter report, and another from a different group, also described the cloning of the human Mec1 and Rad3 counterpart (Bentley et al., 1996; Cimprich et al., 1996). One named it FRAP-related protein 1 (FRP1) because of its similarity to FRAP, another PI3K-related kinase now known as mTOR (Cimprich et al., 1996), while the second named it ATM- and Rad3-related (ATR) (Bentley et al., 1996). Both reports suggested FRP1/ATR (hereafter ATR) to be the ortholog of Mec1 and Rad3, based on sequence similarity and the fact that the ATR cDNA complemented the radiation hypersensitivity of *mec1* budding yeast mutants (Bentley et al., 1996; Cimprich et al., 1996).

With the discovery of ATR, the last of the three vertebrate DDR PIKKs had been identified, and multiple lines of scientific enquiry had converged. Below, we summarize key features of the subsequent two decades of frenzied research, in particular concentrating on the most well-established functions of ATM, ATR, and DNA-PK in the DDR.

Common Features of ATM, ATR, and DNA-PKcs
Domain Organization and Structural Similarities

ATM, ATR, and DNA-PKcs are huge polypeptides with similar domain organizations and various common structural features (Figure 1A). In all cases, their kinase domains are C-terminally located, flanked upstream by a FRAP-ATM-TRRAP (FAT) domain and downstream by a PIKK regulatory domain (PRD) and FAT C-terminal (FATC) motif (Bosotti et al., 2000; Mordes et al., 2008). N-terminal to the FAT domain, all PIKKs consist largely of helical solenoid HEAT-repeat domains of variable
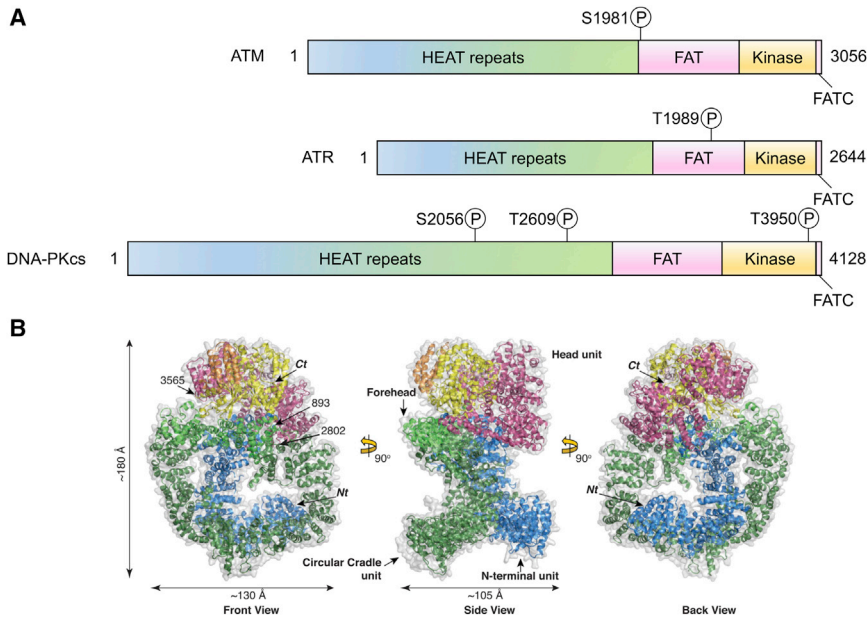
A

B

Figure 1. PIKK Structure
(A) PIKK domain organization. Colored boxes represent indicated protein domains; numbers represent amino acid residues. Major PIKK phosphorylation sites are indicated with an encircled letter P. HEAT, huntingtin, elongation factor 3, A subunit of protein phosphatase 2A, TOR1; FAT, FRAP-ATM-TRRAP; FATC, FAT C-terminal.
(B) Crystal structure of DNA-PKcs. Subdomains are colored as follows: N terminus, blue; circular cradle, green; FAT domain, pink; kinase domain, yellow; FATC domain, light pink. Reproduced with permission from (Sibanda et al., 2017).

length that mediate protein-protein interactions (Perry and Kleckner, 2003).

Due to their large size, limited three-dimensional structural information is available on full-length ATM, ATR, and DNA-PKcs, with the highest resolution crystal structure for these currently being of DNA-PKcs, at 4.3 Å (Sibanda et al., 2017). This structure revealed that DNA-PKcs consists of three large structural units: an N-terminal unit, a central "circular cradle" structure formed by HEAT repeats, and the C-terminal kinase "head" domain (Figure 1B). Single-particle electron microscopy (EM) studies of Mec1^{ATR} and Tel1^{ATM} suggest that these kinases form similar architectures, with the N-terminal HEAT repeats fashioning a possible cradle region and the FAT-kinase-FATC domains forming a head region (Sawicka et al., 2016).

Substrate Specificity
Early biochemical studies on DNA-PK revealed its preference for phosphorylating a serine or threonine residue followed by a glutamine (S/T-Q; Bannister et al., 1993; Chen et al., 1991; Lees-Miller and Anderson, 1989). This was later shown to be the case for ATM and ATR as well (Kim et al., 1999). Furthermore, these studies showed that hydrophobic or acidic residues at positions immediately flanking the phosphorylated serine/threonine tend to be positive determinants for targeting, whereas basic residues in their vicinity are generally negative determinants. In line with this, DNA-PK, ATM, and ATR share certain substrates and have some overlapping functions, as we discuss in later sections. It should be noted, however, that DNA-PK has also been shown to target Ser/Thr residues within non-S/T-Q contexts in vitro (Jette and Lees-Miller, 2015).

Autophosphorylation
Various S/T-Q motifs are present in ATM, ATR, and DNA-PKcs, and all three can autophosphorylate. For DNA-PKcs, multiple autophosphorylation sites have been identified, which mostly cluster in the HEAT-repeat region (Jette and Lees-Miller, 2015). Autophosphorylation of S2056 and T2609 in particular is used to indi-

cate DNA-PKcs activation in human cells (Chan et al., 2002; Chen et al., 2005), although there is some dispute as to whether T2609 may also be phosphorylated by ATM and ATR in certain circumstances (Chen et al., 2007; Jiang et al., 2015; Meek et al., 2007; Yajima et al., 2006). Neither S2056 nor T2609 is required for DNA-PKcs kinase activity, but both are important for DNA repair, with current models suggesting that their phosphorylation causes conformational changes that promote DNA-PK disassembly from DSB sites to allow DNA-end ligation (Jette and Lees-Miller, 2015). This is supported by the recent crystal structure of DNA-PKcs in complex with a Ku80 peptide, which suggests that S2056 phosphorylation may regulate Ku80 binding (Sibanda et al., 2017). A third autophosphorylation site, T3950, is located in the kinase domain and may act to switch off DNA-PKcs kinase activity when phosphorylated (Douglas et al., 2007; Sibanda et al., 2017).

For ATM and ATR, roles for autophosphorylation are more controversial. Human ATM phosphorylates itself on S1981, an event proposed to transition the kinase from an inactive dimer into active monomers in response to DNA damage (Bakkenist and Kastan, 2003). While experiments with A-T cells complemented with ATM autophosphorylation mutants, including S1981A, supported this model (Kozlov et al., 2006), subsequent studies found that S1981 does not affect ATM activation in vitro and can be uncoupled from ATM monomerization (Dupré et al., 2006; Lee and Paull, 2005). Furthermore, mice expressing mutant ATM that cannot be phosphorylated on this residue (and on additional autophosphorylation sites) are phenotypically normal with no observed defects in ATM-dependent responses (Daniel et al., 2008; Pellegrini et al., 2006). A recent single-particle EM structure of dimeric human ATM also does not support a role for S1981 in ATM activation (Lau et al., 2016). Further work is clearly needed to elucidate the functions of S1981 and other ATM autophosphorylation events.

Like ATM and DNA-PKcs, ATR autophosphorylates in vitro (Cliby et al., 1998). One ATR autophosphorylation site is T1989 (Liu et al., 2011; Nam et al., 2011), although there is disagreement about whether T1989 plays a major role in ATR function. T1989 is located in the highly conserved ATR FAT domain, but the site itself and surrounding residues are not well conserved, even in mammals. Nevertheless, this site can be a useful marker for ATR activation. Commercial antibodies are also available
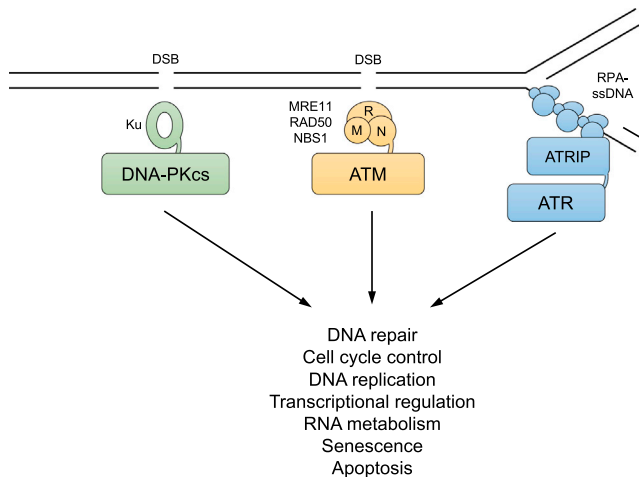
Figure 2. Model for PIKK Recruitment and Activation in Response to DNA Damage
DNA-PKcs is recruited and activated by Ku-bound DSB ends. ATM is activated and recruited to DSBs by the MRE11-RAD50-NBS1 (MRN) complex. ATR is recruited to RPA-coated ssDNA by its stable binding partner ATRIP.

against another phosphorylation site, S428, in the ATR N-terminal region, but this site is not induced upon ATR activation nor required for ATR-dependent phosphorylation events (Liu et al., 2011; Nam et al., 2011), so caution should be employed before using this mark as a readout of ATR activity.

Recruitment to DNA Damage Sites
ATM, ATR, and DNA-PKcs must be tightly regulated to prevent aberrant activation that could lead to toxic DNA repair, cell-cycle arrest, senescence, or apoptosis. Indeed, each kinase requires a specific protein co-factor for stable recruitment to DNA damage sites (Figure 2): this is NBS1 for ATM (Falck et al., 2005), ATRIP for ATR (Zou and Elledge, 2003), and Ku80 for DNA-PKcs (Gell and Jackson, 1999; Singleton et al., 1999). Although each kinase requires a distinct factor, a common principle applies to their recruitment because NBS1, ATRIP, and Ku80 all share a related C-terminal motif required for PIKK binding, probably via interactions with the PIKK HEAT repeats (Ball et al., 2005; Falck et al., 2005; Spagnolo et al., 2006).

DNA-PKcs, Regulator of DNA Repair by NHEJ
Mechanism of DNA-PKcs Activation and Its Function in Cells
DNA-PKcs is activated when it is recruited to DSBs by Ku, as described above. The crystal structure of the Ku heterodimer revealed it to be basket shaped, with a central cavity that accommodates a dsDNA end (Walker et al., 2001). DNA-PK thus acts as a sensor for DSBs, and its major role is to promote NHEJ (Jette and Lees-Miller, 2015). NHEJ repairs most DSBs in mammalian cells, except when they occur at DNA replication forks when homologous recombination (HR) is preferentially used (Beucher et al., 2009; Karanam et al., 2012). As its name implies, NHEJ involves ligation of two broken DNA ends without needing a repair template. Although classical NHEJ is often described as error prone and mutagenic (for example in the context of gene editing by CRISPR-Cas9 [Hsu et al., 2014]), in reality it is remarkably effi-

cient and mostly accurate (Bétermier et al., 2014). Only when classical NHEJ fails must cells use an alternative end-joining pathway such as that mediated by DNA polymerase θ (POLQ), which can introduce extensive mutations (van Schendel et al., 2015).

Mechanistically, NHEJ is initiated by Ku and DNA-PKcs binding to DSBs, with the ensuing DNA-PK holoenzyme promoting DNA-end tethering (Graham et al., 2016). Additional NHEJ core factors are subsequently recruited for the ends to be closely aligned and ligated, including XRCC4 (Li et al., 1995), XLF (Ahnesorg et al., 2006; Buck et al., 2006), and DNA ligase IV (LIG4; Critchlow et al., 1997; Grawunder et al., 1997). The NHEJ machinery including DNA-PKcs is stabilized on damaged chromatin by PAXX (Ochi et al., 2015), a second XRCC4-like core NHEJ factor (Figure 3).

Some DSBs repaired by NHEJ require additional accessory proteins such as DNA polymerases and nucleases for their repair. One such factor is the endonuclease Artemis (Moshous et al., 2001), which interacts with DNA-PKcs to promote repair of a subset of DSBs (Ma et al., 2002; Riballo et al., 2004; Rooney et al., 2003). Indeed, in addition to its end-tethering role, a major function of DNA-PKcs is to recruit Artemis to promote DNA-end processing (Jiang et al., 2015; Malu et al., 2012).

DNA-PKcs kinase activity is important for NHEJ, and various putative DNA-PK substrates have been reported, including other NHEJ factors (Jette and Lees-Miller, 2015). However, it is not yet clear whether NHEJ requires DNA-PKcs to phosphorylate any proteins other than itself. While DNA-PK inhibition does not block recruitment of downstream factors such as LIG4 (Jiang et al., 2015), it does prevent formation of short-range synaptic complexes (Graham et al., 2016). This indicates that DNA-PKcs is a physical block to NHEJ and that autophosphorylation relieves this to allow DNA-end alignment and completion of repair.

Physiological Roles of DNA-PKcs in Development
During lymphocyte development, DSBs are generated in immunoglobulin and T cell receptor loci to generate immune-receptor diversity by V(D)J and class-switch recombination (Alt et al., 2013). As such DSBs require NHEJ for efficient repair, defects in most core NHEJ factors cause severe immunodeficiency in mammals. PAXX and XLF are exceptional in this regard, as both must be simultaneously lost to produce substantial immune defects in mice due to their partial functional redundancy in B cells (Balmus et al., 2016; Kumar et al., 2016; Lescale et al., 2016; Liu et al., 2017). Like PAXX, ATM is also crucial for V(D)J recombination in the absence of XLF, as discussed below.

In addition to abrogating B and T cell development, specific mutations in DNA-PKcs that inhibit its kinase activity also cause profound neurological defects (Jiang et al., 2015; Woodbine et al., 2013), phenotypes that are also associated with deficiencies in certain other NHEJ components (Balmus et al., 2016; Gao et al., 1998; Saito et al., 2016). While this suggests that NHEJ factors play important roles in repairing DSBs that arise during differentiation of neural progenitor cells, the nature and etiology of such DSBs remain to be determined.

ATM, Master Regulator of Cellular Responses to DSBs
ATM Activates a DSB-Signaling Cascade
ATM is the apical kinase responsible for global orchestration of cellular responses to DSBs, which include DNA repair,
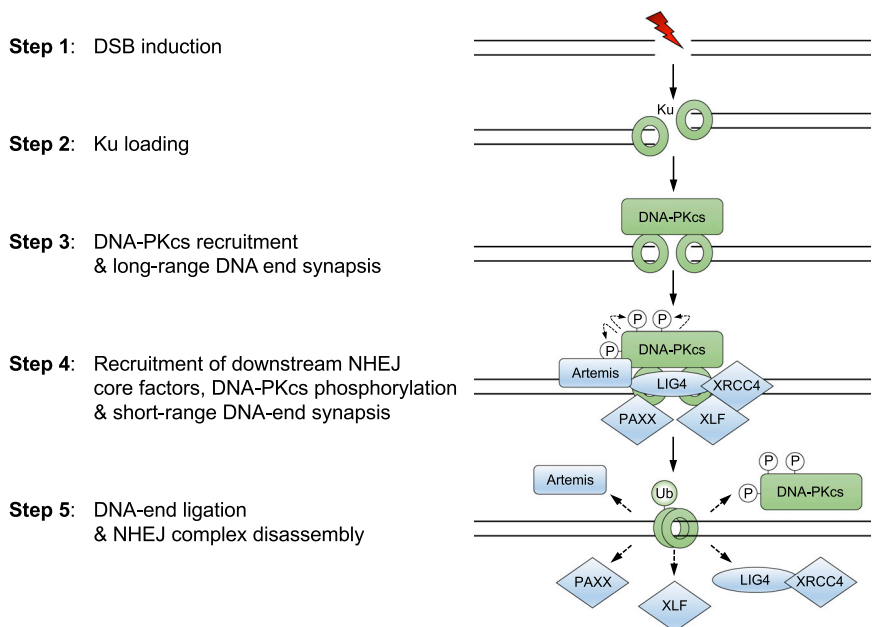
Step 1:   DSB induction

Step 2:   Ku loading

Step 3:   DNA-PKcs recruitment
& long-range DNA end synapsis

Step 4:   Recruitment of downstream NHEJ
core factors, DNA-PKcs phosphorylation
& short-range DNA-end synapsis

Step 5:   DNA-end ligation
& NHEJ complex disassembly

Figure 3. Model for the Role of DNA-PK in NHEJ
DNA-PK (green) is recruited and activated by Ku-bound DSB ends to promote NHEJ. Downstream NHEJ factors are colored blue. XRCC4, XLF, and PAXX (diamond shapes) are structurally related paralogs. Note that this model does not reflect the relative numbers of NHEJ factors that may be present at DSBs.

checkpoint activation, apoptosis, senescence, and alterations in chromatin structure, transcription, and pre-mRNA splicing (Shiloh and Ziv, 2013). To achieve this, ATM phosphorylates hundreds of substrates in response to DNA damage (see for example Matsuoka et al., 2007), although it is unclear what proportion of these is functionally important. Most ATM substrates are probably also phosphorylated by ATR in response to replication stress and a few, such as histone H2AX, by DNA-PKcs as well. Notably, ATM phosphorylates and activates other protein kinases that phosphorylate yet more substrates, meaning that ATM-dependent signaling events are not just restricted to factors directly phosphorylated by ATM (Shiloh and Ziv, 2013). A well-characterized example of this is the CHK2 kinase, which is activated by ATM and phosphorylated on multiple sites including T68 (Ahn et al., 2000; Brown et al., 1999; Matsuoka et al., 1998, 2000; Melchionna et al., 2000). While CHK2-T68 phosphorylation is routinely used to indicate ATM activation, it is worth noting that this modification may not be solely ATM-dependent in all circumstances (Matsuoka et al., 2000). Checkpoint signaling is largely intact in CHK2-deficient cells, but they are resistant to IR, highlighting roles for ATM and CHK2 in triggering apoptosis (Hirao et al., 2002; Jack et al., 2002; Takai et al., 2002).

Activation of p53 by ATM
The tumor suppressor p53 is stabilized in response to DNA damage to activate a transcriptional program that can lead to cell-cycle arrest in G1, senescence, or apoptosis (Shiloh and Ziv, 2013). ATM can phosphorylate p53 on multiple sites, with modification of S15 having been particularly well studied (Banin et al., 1998; Canman et al., 1998; Siliciano et al., 1997). S15 phosphorylation was proposed to inhibit interaction of p53 with the ubiquitin ligase MDM2, resulting in rapid p53 stabilization and activation in response to DSBs (Shieh et al., 1997); however, subsequent studies have questioned this model and indicated that ATM-

dependent phosphorylation of MDM2 may be more important for p53 stabilization (Cheng and Chen, 2010). It is important to note, however, that ATM controls p53 stability via multiple mechanisms by phosphorylating not just p53 itself but also other proteins that directly or indirectly influence p53 stability (Shiloh and Ziv, 2013). One such protein is the p53 co-factor hnRNP K, which is phosphorylated by ATM in response to DSBs and protected from MDM2-mediated degradation (Moumen et al., 2013, 2005). As with various ATM-dependent processes, ATR can activate similar pathways in response to other types of genotoxic stress (Lakin et al., 1999; Moumen et al., 2005; Tibbetts et al., 1999).

Mechanism of ATM Activation
ATM is recruited to chromatin in response to DSBs (Andegeko et al., 2001; Bekker-Jensen et al., 2006), in a process that requires ATM binding to the C terminus of NBS1 (Falck et al., 2005), a component of the MRE11-RAD50-NBS1 (MRN) complex (Carney et al., 1998). This complex can stimulate ATM kinase activity directly in vitro (Lee and Paull, 2004, 2005), and is required for optimal ATM signaling in cells (Carson et al., 2003; Uziel et al., 2003), indicating that MRN both recruits ATM to DNA lesions and stimulates ATM kinase activity once there. However, the exact mechanism whereby MRN activates ATM is still not understood. MRN/DSB-independent means of ATM activation also exist, for example in response to oxidative stress or chromatin changes (Guo et al., 2010; Kaidi and Jackson, 2013; Olcina et al., 2013).

Early biochemical studies with immunoprecipitated endogenous ATM from cell extracts demonstrated that pre-treating cells with DSB-inducing agents results in a more active ATM kinase (Banin et al., 1998; Canman et al., 1998). This may be explained, at least in part, by ATM autophosphorylation, as well as the existence of regulatory mechanisms promoting ATM activation following DSB-associated chromatin changes. Upon DNA damage, exposed nucleosomes bearing the H3K9me3 histone mark are recognized by the TIP60/KAT5 acetyltransferase (Sun et al., 2009), which is thus recruited to damaged chromatin and shielded from dephosphorylation (Kaidi and Jackson, 2013). Phosphorylated TIP60 is activated and stimulates ATM by acetylating its FATC motif on K3016 (Sun et al., 2005, 2007). This process may be promoted by FOXO3A and inhibited by NOTCH1, which compete for binding to the ATM C terminus (Adamowicz et al., 2016). Notably, the enzyme mediating
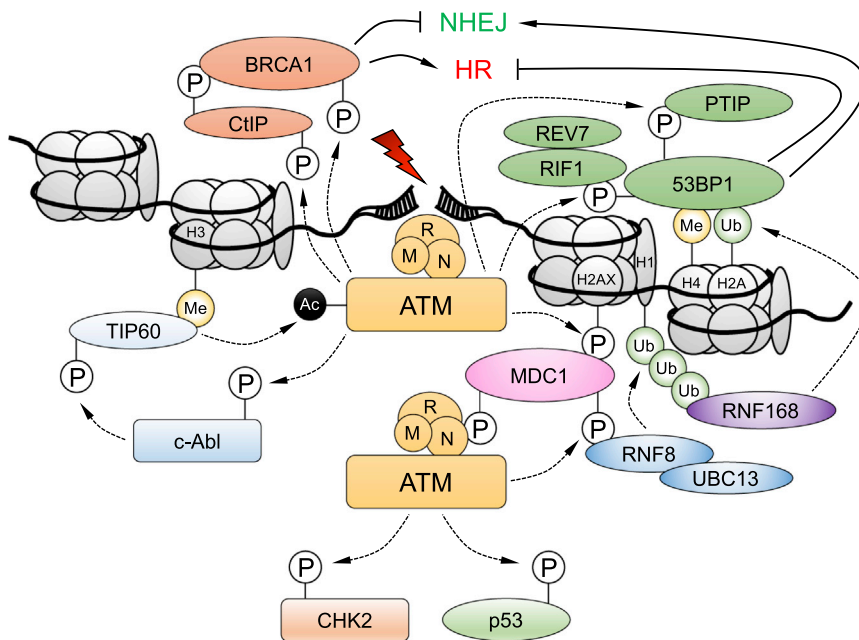
TIP60 phosphorylation is the c-Abl tyrosine kinase (Kaidi and Jackson, 2013), which is itself phosphorylated and activated by ATM in response to DNA damage (Baskaran et al., 1997; Shafman et al., 1997). These data thus suggest the existence of a positive feedback loop that reinforces ATM activation.

Roles of ATM in DSB Repair

While most (~80%) IR-induced DSBs outside of S phase are repaired by NHEJ independently of ATM (Beucher et al., 2009), a minority is repaired by a pathway requiring ATM, DNA-PK, the MRN complex, and Artemis (Riballo et al., 2004). This subset may include DSBs in heterochromatic regions and those with blocked ends that require processing (Álvarez-Quilón et al., 2014; Goodarzi et al., 2008). Mechanistically, MRN-dependent ATM activation may trigger phosphorylation of DNA-PKcs to promote Artemis recruitment and activation (Goodarzi et al., 2006; Jiang et al., 2015), a model supported by the demonstration that Ku and MRN can simultaneously localize to the same DSB site in cells (Britton et al., 2013). Together with PAXX, ATM plays at least one important additional role in NHEJ that is not yet clear but which is redundant with XLF and is therefore not normally apparent (Balmus et al., 2016; Zha et al., 2011). Given that ATM substrates on chromatin involved in DNA-end protection and bridging, such as H2AX and 53BP1, also share redundant functions with XLF in NHEJ, it is possible that the role of ATM in NHEJ relates to DSB-end bridging (Kumar et al., 2014b).

ATM also promotes HR, the other major DSB repair pathway. HR requires DSB-end processing to produce tracts of single-stranded DNA (ssDNA) that, once bound by the recombinase RAD51, invade another DNA molecule (normally a sister chromatid) that serves as a repair template (Kowalczykowski, 2015). This process, termed DNA-end resection, is a key determinant of DSB repair pathway choice (Chapman et al., 2012) and is stimulated by ATM (Adams et al., 2006; Cuadrado et al., 2006; Jazayeri et al., 2006; Myers and Cortez, 2006). In part,

this stimulation occurs via ATM directly phosphorylating the MRN-interacting protein CtIP, a key factor required for resection initiation (Sartori et al., 2007; Shibata et al., 2011; Wang et al., 2013). ATM-mediated CtIP phosphorylation may also impact on later stages of HR, as it promotes the removal of Ku from single-ended DSB ends at collapsed replication forks to allow the strand invasion step of HR to proceed (Britton et al., 2013; Chanut et al., 2016). Despite these connections, it is important to note that ATM is not essential for HR and that HR-driven processes can take place in its absence.

ATM Initiates and Sustains Chromatin-Based DDR Signaling

DSBs trigger rapid phosphorylation of S139 in the C-terminal tail of histone variant H2AX (Rogakou et al., 1998). This modification, termed γH2AX, is primarily mediated by ATM in response to DSBs and forms the foundation of a chromatin-based signaling cascade involving phosphorylation, ubiquitylation, and other post-translational modifications (Scully and Xie, 2013) (Figure 4). The major reader of the γH2AX mark is MDC1, which possesses C-terminal tandem BRCT domains that specifically bind γH2AX at DSB sites (Stucki et al., 2005). There, MDC1 is stabilized on chromatin by ATM, which phosphorylates MDC1 on T4 to promote MDC1 dimerization via its FHA domain (Jungmichel et al., 2012; Liu et al., 2012). MDC1 is constitutively phosphorylated by CK2 on multiple other residues, which are recognized by the FHA-BRCT region of NBS1 to promote MDC1-MRN retention on γH2AX-containing chromatin (Chapman and Jackson, 2008; Melander et al., 2008; Spycher et al., 2008). An attractive model is that MRN recruitment by MDC1 leads to further recruitment of ATM via its interaction with NBS1, leading to additional γH2AX formation and MDC1-MRN-ATM recruitment, thus spreading the assembly along chromatin and amplifying DDR signaling.

At DSB sites, ATM phosphorylates MDC1 on T-Q-X-F motifs that are recognized by the FHA domain of the ubiquitin ligase RNF8, thus promoting RNF8 retention on damaged chromatin (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007). With the ubiquitin-conjugating enzyme UBC13, RNF8 stimulates ubiquitylation of the linker histone H1 (Thorslund et al., 2015). Ubiquitylated H1 is recognized by ubiquitin-binding domains in

another ubiquitin ligase, RNF168, which ubiquitylates K15 of H2A-type histones to promote recruitment of the scaffold protein 53BP1 to channel DSB repair toward NHEJ (Fradet-Turcotte et al., 2013; Mattiroli et al., 2012; Thorslund et al., 2015). In turn, 53BP1 recruits further proteins by mechanisms that involve ATM. One such factor, PTIP, associates directly with the 53BP1 extreme N terminus in a manner requiring PTIP BRCT domains and ATM-dependent phosphorylation of 53BP1 on S25 (Jowsey et al., 2004; Muñoz et al., 2007). A second, RIF1, interacts with 53BP1 independently of PTIP but in a manner requiring phosphorylation of the 53BP1 N terminus by ATM; ensuing 53BP1 complexes containing PTIP, RIF1, REV7/MAD2L2, and other factors promote DSB repair by NHEJ through mechanisms that are not yet clear (Hustedt and Durocher, 2016).

Some 53BP1 functions are antagonized by BRCA1, the product of a highly penetrant breast and ovarian tumor suppressor gene (Miki et al., 1994). ATM, the loss of which also predisposes to breast cancer (Swift et al., 1987), functionally phosphorylates BRCA1 on multiple residues in response to DNA damage (Cortez et al., 1999). BRCA1 exists in several protein complexes with discrete functions in maintaining genome stability as well as other processes, but its major function seems to be to promote HR at the expense of 53BP1-mediated NHEJ (Hustedt and Durocher, 2016). The complex mechanisms whereby 53BP1 and BRCA1 assemblies counteract each other and otherwise function to maintain genome integrity—and how such processes are regulated by ATM and subject to cell-cycle control (e.g., Orthwein et al., 2015)—are still emerging and beyond the scope of this review.

Notably, γH2AX-marked chromatin is transcriptionally inactive (Chou et al., 2010; Polo et al., 2012), possibly to help prevent collisions between DNA repair and transcription machineries. Such transcriptional silencing may also counteract formation of DNA-RNA hybrids such as R loops (Britton et al., 2014). Depending on its cellular context, transcriptional silencing can depend on ATM, ATR, or DNA-PKcs (Britton et al., 2014; Pankotai et al., 2012; Shanbhag et al., 2010), with ATM being particularly important for nucleolar DNA silencing (Kruhlak et al., 2007). Mechanistically, ATM-dependent transcriptional silencing of RNA polymerase II depends on phosphorylation of the BAF180 subunit of the PBAF SWI/SNF chromatin remodeling complex (Kakarougkas et al., 2014).

Another class of ATM- and H2AX-dependent, transcriptionally repressed chromatin foci are 53BP1-OPT domains, which form specifically in G1 phase cells around DNA lesions carried over from the previous cell cycle (Harrigan et al., 2011; Lukas et al., 2011). OPT domains disappear as cells enter S phase and may contain under-replicated DNA, which is shielded from end processing by 53BP1 until the next round of DNA replication starts.

Other roles for γH2AX foci may be to concentrate factors in "liquid-demixed" zones, where DSB clustering may enhance DNA repair efficiency (Altmeyer et al., 2015; Caron et al., 2015). γH2AX chromatin domains might also define boundaries to avert over-resection of DSBs and counteract limitations in repair-factor supply (Ochs et al., 2016). Importantly, while ATM predominates in generating γH2AX under many circumstances, in some contexts, ATR or DNA-PKcs can substitute partly or entirely. For example, during male mammalian meiosis, ATR-mediated γH2AX generation globally represses almost all transcription on the non-recombining X-Y chromosome pair (Royo et al., 2013).

ATR, an Essential Kinase Involved in DNA Replication Stress Responses

ATR Activation Is a Multi-step Process

ATR is the apical DNA replication stress response kinase, phosphorylating many substrates in response to agents such as UV (see for example Matsuoka et al., 2007). Unlike ATM and DNA-PKcs, ATR is essential in proliferating cells (Brown and Baltimore, 2000; de Klein et al., 2000), which hampered efforts to study its functions until the recent development of selective small-molecule ATR kinase inhibitors (Fokas et al., 2014). Nonetheless, previous work using *Xenopus* egg extracts, RNA interference, or cells expressing hypomorphic ATR variants has been highly informative. One such cell type is derived from patients with Seckel syndrome, a hereditary form of microcephalic dwarfism caused by an *ATR* pre-mRNA splicing mutation that results in very low ATR protein levels (O'Driscoll et al., 2003).

In contrast to ATM and DNA-PKcs, which respond primarily to DSBs, ATR is activated by a much wider range of genotoxic stresses. This is because ATR is recruited, via its partner protein ATRIP, to extended tracts of ssDNA coated with the ssDNA-binding protein complex, replication protein A (RPA) (Zou and Elledge, 2003) (Figure 5). Such RPA-coated ssDNA is generated by nucleolytic processing of various forms of damaged DNA (Raderschall et al., 1999) or by helicase-polymerase uncoupling at stalled replication forks (Byun et al., 2005).

ATR recruitment to RPA-ssDNA is not sufficient for optimal activation but also requires the presence of activator proteins and ssDNA/dsDNA junctions. The best-characterized ATR activator is TopBP1, which contains an ATR-activation domain that stimulates ATR kinase activity (Kumagai et al., 2006) via contacts with both ATRIP and the C-terminal PRD in ATR (Mordes et al., 2008). As with ATR itself, TopBP1 loss or an inactivating mutation in the TopBP1 ATR-activation domain is lethal in mammalian cells (Jeon et al., 2011; Zhou et al., 2013). One important interaction of TopBP1 is its binding to the C-terminal tail of the RAD9 subunit of the RAD9-RAD1-HUS1 (9-1-1) complex (Delacroix et al., 2007; Lee et al., 2007), a ring-shaped heterotrimer that is loaded onto RPA-ssDNA/dsDNA junctions by the RAD17-RFC complex (Ellison and Stillman, 2003; Zou et al., 2002, 2003). However, while the TopBP1-RAD9 interaction is important for ATR activation, it is not essential for TopBP1 recruitment to stalled replication forks (Lee and Dunphy, 2010; Yan and Michael, 2009), suggesting that this must be mediated by other, potentially overlapping, mechanisms. In line with this, other factors including MRN and BLM have been implicated in TopBP1 recruitment (Blackford et al., 2015; Duursma et al., 2013); but, it is likely that TopBP1 localization to damaged DNA is complex, requiring numerous protein-protein interactions influenced by cell-cycle stage and genotoxic insult.

Recently, a second ATR-activator protein, ETAA1, was identified and found to contain an ATR-activation domain similar to that of TopBP1 (Bass et al., 2016; Feng et al., 2016; Haahr et al., 2016). Unlike TopBP1, ETAA1 is recruited to RPA-ssDNA via direct binding to RPA. It is thus tempting to speculate that
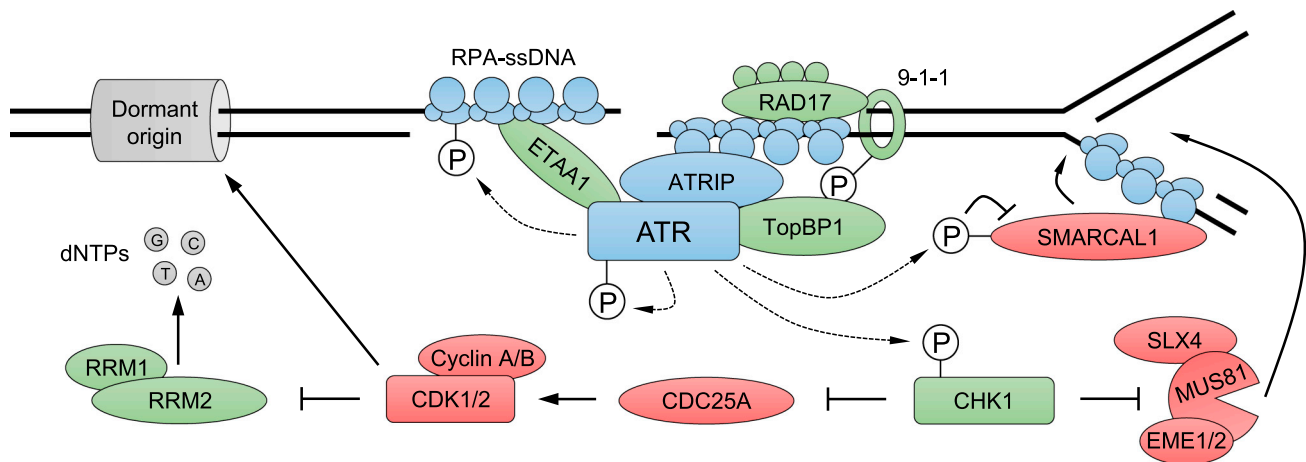
Figure 5. ATR Recruitment and Activation of S Phase Checkpoint Signaling
ATR is recruited to RPA-ssDNA and activated by TopBP1 or ETAA1. ETAA1 is recruited directly to RPA-coated ssDNA. TopBP1 is recruited via mechanisms that are not yet clear, but its role in ATR activation requires interaction with the RAD9-HUS1-RAD1 (9-1-1) clamp complex, which is loaded onto ds/ssDNA junctions by the RAD17/RFC2-5 clamp loader. ATR signaling activates the CHK1 kinase and restrains fork processing enzymes such as SMARCAL1. CHK1 activation causes CDC25A degradation, leading to inhibition of CDK activity, slowing of cell-cycle progression, inhibition of late-origin firing, and increased nucleotide availability, in part by upregulation of RRM2. ATR and its recruitment factors are shown in blue. Factors with a positive role in ATR stimulation or that are activated by ATR are in green, while those that are inhibited by ATR are in red. See text for further details.

TopBP1 and ETAA1 may be recruited to different types of aberrant DNA structures to stimulate ATR toward discrete sets of substrates.

Roles for ATR in Checkpoint Signaling
A key ATR function is to phosphorylate and activate the protein kinase CHK1 (Guo et al., 2000; Hekmat-Nejad et al., 2000; Liu et al., 2000; Zhao and Piwnica-Worms, 2001). When activated, CHK1 promotes proteasomal degradation of CDC25A, a phosphatase that removes inhibitory modifications from cyclin-dependent kinases (CDKs) (Bartek et al., 2004). CDC25A inactivation by CHK1 in response to genotoxic stress thereby slows or arrests cell-cycle progression by reducing CDK activity. This presumably allows more time for DNA repair so that cells do not enter mitosis prematurely or, if the damage is too extensive, for activation of senescence or apoptotic pathways. The importance of the ATR-CHK1-CDC25A axis for cell survival is highlighted by the lethality associated with ATR or CHK1 inhibition being circumvented by CDC25A inactivation (Carette et al., 2011; Ruiz et al., 2016).

In addition to preventing premature mitotic entry, it was previously thought that ATR played a role in regulating replisome stability (Cortez, 2015). However, recent data using methods that allow observation of global replisome stability genome wide suggest that in both human and yeast cells, the replisome is not disassembled when cells lacking ATR/Mec1 experience replicative stress (De Piccoli et al., 2012; Dungrawala et al., 2015). Instead, ATR may prevent replication fork collapse primarily through the following mechanisms. First, by inhibiting CDKs, ATR signaling restrains replication origin firing (Costanzo et al., 2003). This is important to avoid exhaustion of replication and repair factor pools, particularly RPA (Toledo et al., 2013). Second, ATR limits replication fork collapse by directly targeting helicases such as SMARCAL1, which can produce fork configurations that are recognized and cleaved by SLX4/MUS81 nuclease complexes

(Couch et al., 2013; Forment et al., 2011; Ragland et al., 2013). Finally, ATR regulates deoxyribonucleotide availability in mammalian cells by promoting upregulation of the ribonucleotide reductase subunit RRM2 at the transcriptional and post-translational levels in response to DNA damage (Buisson et al., 2015; D'Angiolella et al., 2012; Pfister et al., 2015). This is highlighted by studies showing that an extra copy of *RRM2* protects against some of the genome instability associated with ATR loss in mice (López-Contreras et al., 2015).

ATR Activates the Fanconi Anemia Pathway
While ATR phosphorylates many of the same substrates as ATM (Matsuoka et al., 2007), it may also have unique targets—particularly those functioning in pathways connected to replication fork repair. One of these is the Fanconi anemia (FA) pathway, which promotes repair of DNA interstrand crosslinks (ICLs) (Ceccaldi et al., 2016). FA is a rare hereditary genetic disorder characterized by bone marrow failure and cancer predisposition, with some patients also displaying microcephaly and dwarfism features reminiscent of ATR-Seckel syndrome. These common characteristics may reflect a requirement for ATR in ICL repair by promoting FANCD2 mono-ubiquitylation, a key step in the FA pathway (Andreassen et al., 2004). This is probably achieved via phosphorylation of FANCI by ATR (Ishiai et al., 2008), an event that may also play a more general role in the replication stress response by regulating dormant origin firing (Chen et al., 2015).

Cross-Talk among ATM, ATR, and DNA-PKcs
The G1/S, intra-S, and G2/M checkpoints induced by DNA damage signaling are similar in that the endpoint of all three is to inhibit CDK activity. DSBs are not resected to generate significant amounts of RPA-ssDNA in G1 (Jazayeri et al., 2006), so the G1/S checkpoint is believed to be controlled primarily by ATM rather than ATR. By contrast, both ATM and ATR contribute to establishment and maintenance of the intra-S and G2/M

checkpoints, in part because ATM-dependent DNA-end resection provides the RPA-ssDNA signal for ATR recruitment and activation (Adams et al., 2006; Cuadrado et al., 2006; Jazayeri et al., 2006; Myers and Cortez, 2006). This ATM-dependent ATR activation is one example of functionally important crosstalk between PIKKs. As discussed earlier, ATM- and ATR-dependent phosphorylation of DNA-PKcs may be important for DNA repair, and recent evidence suggests that DNA-PK also phosphorylates ATM to restrain its activity (Zhou et al., 2017). Furthermore, ATR has been reported to phosphorylate ATM in response to UV exposure (Stiff et al., 2006).

Additional Biological Roles for ATM, ATR, and DNA-PKcs
Activation in Response to Telomere Dysfunction
Under normal circumstances, chromosome ends do not trigger DNA damage signaling, as telomeric DNA is bound by the shelterin complex, which inhibits ATM, ATR, and DNA-PK via multiple mechanisms (de Lange, 2009). For instance, shelterin component TRF2 promotes formation of a T-loop structure that sequesters the telomeric DNA end, thereby blocking MRN and Ku binding and inhibiting ATM and DNA-PK activities, respectively. Furthermore, the POT1 component of shelterin binds telomeric ssDNA, thus excluding RPA and preventing ATR recruitment. In cells lacking telomerase, telomeres become progressively shorter after each DNA replication cycle, eventually leading to telomere dysfunction and ATM/ATR-mediated cell-cycle arrest, followed by senescence (d'Adda di Fagagna et al., 2003).

Connections between ATM and Transcriptional Stress
Regulators of RNA metabolism are frequently identified in screens for DDR components (e.g., Paulsen et al., 2009). Some appear to have dual roles in transcription and DNA repair (Polo et al., 2012), while others may be targeted by PIKKs to modulate RNA metabolism. Indeed, emerging evidence suggests the spliceosome is both a target and an effector for ATM in response to R-loop formation (Tresini et al., 2015). Interestingly, the helicase senataxin, which promotes R-loop resolution (Skourti-Stathaki et al., 2011), is mutated in ataxia-ocular apraxia 2 (Moreira et al., 2004). This form of ataxia is reminiscent of A-T, but patients are not radiosensitive and do not get lymphoid cancers. These findings raise the possibility that at least some neurological phenotypes associated with ATM deficiency may be linked to defective resolution of DNA-RNA hybrids.

ATR as a Sensor of Mechanical Forces
Chromatin dynamics generate mechanical forces that can be transmitted to the nuclear envelope via lamin-associated chromatin domains. In yeast, Mec1^{ATR} facilitates DNA replication by releasing such domains from the nuclear envelope as replication forks approach them, thereby avoiding generation of aberrant DNA topology that could lead to fork reversal (Bermejo et al., 2011). ATR is also activated at the nuclear envelope in human cells in response to mechanical stress in a manner apparently independent of RPA (Kumar et al., 2014a). Intriguingly, HEAT repeats, such as those in PIKKs, can act as elastic connectors (Grinthal et al., 2010), suggesting that ATR and possibly other PIKKs may sense mechanical forces directly.

Connections between PIKKs and Viral Infection
Viruses cause various human diseases and are significant contributors to cancer (Weitzman and Weitzman, 2014). Viral genomes and their replication intermediates can resemble damaged DNA to the host cell, which could trigger DDRs that might limit the productivity of viral infection. Presumably as a consequence, many viruses encode proteins that specifically inhibit ATM, ATR, or DNA-PK activity, either completely or partially. A common strategy is to hijack cellular ubiquitylation pathways to target PIKKs (Lees-Miller et al., 1996) or their activator proteins such as TopBP1 and the MRN complex (Blackford et al., 2010; Stracker et al., 2002), for proteasomal degradation.

Small Molecule Inhibitors of ATM, ATR, and DNA-PK as Potential Therapeutic Agents
As the DDR can be activated at early stages in tumorigenesis due to oncogene-induced replication stress or processes such as telomere shortening, this has suggested that cell-cycle arrest or cell death enforced by PIKK signaling may serve as a barrier to tumorigenesis (Bartkova et al., 2005, 2006; d'Adda di Fagagna et al., 2003; Gorgoulis et al., 2005). In line with this, ATM is frequently mutated in human cancers (Macheret and Halazonetis, 2015). Why, then, should inhibiting PIKKs be a promising avenue for cancer treatment? The answer lies in the fact that precisely because rapid, unregulated cell division and genome instability are hallmarks of cancer (Hanahan and Weinberg, 2011), tumor cells are inherently vulnerable to additional replication stress, exogenous DNA damage, or DDR inhibition. This probably in large part explains therapeutic successes with radiotherapy and various DNA-damaging chemotherapeutics and also contributes to the anti-cancer properties of emerging DDR-enzyme inhibitors (O'Connor, 2015). Small molecule inhibitors are now available against all three PIKKs and have entered phase I or II clinical trials both as single agents and in combination with radiotherapy or traditional chemotherapeutics (Brown et al., 2017; O'Connor, 2015). ATR is a particularly attractive target for anticancer therapy, as tumor cells are likely to rely heavily on ATR for survival, due to their high burden of replication stress. This provides both the rationale for the use of ATR inhibitors in multiple types of cancers and provides an explanation for the fact that ATR is not a strong tumor suppressor despite its role in maintaining genome stability (Lecona and Fernández-Capetillo, 2014).

Furthermore, genome instability in many tumors is caused by defects in certain DNA repair pathways, such as HR. This offers the potential for targeted therapies to exploit the concept of synthetic lethality, whereby loss of one cellular pathway results in high reliance on another pathway that is not essential under normal settings. While this is perhaps best exemplified by the use of PARP inhibitors to target HR-deficient tumors (Bryant et al., 2005; Farmer et al., 2005), it is notable that growing evidence highlights how loss of ATM and other DDR factors also leads to PARP-inhibitor sensitivity in cancers (Lord and Ashworth, 2017). Interestingly, and perhaps not surprisingly given their somewhat overlapping functions, synthetic lethal relationships exist between ATM and DNA-PKcs (Gurley and Kemp, 2001; Sekiguchi et al., 2001) and between ATM and ATR (Balmus et al., 2012; Reaper et al., 2011), although apparently not between ATR and DNA-PKcs (Middleton et al., 2015). Such relationships might be exploited therapeutically by specific DDR-PIKK inhibitor drugs in certain cancers deficient in ATM, ATR, or DNA-PK.

Finally, we note that our growing appreciation of DDR-PIKK biology is suggesting therapeutic opportunities in other disease areas. ATM inhibition has, for instance, been suggested as a potential anti-retroviral strategy (Lau et al., 2005). Furthermore, there is a growing realization that hyperactive DDR signaling is associated with various neurodegenerative diseases, as highlighted by recent work showing that ATM inhibition ameliorates pathologies in models of Huntington's disease (Lu et al., 2014).

Conclusions and Future Perspectives

Since their discovery over 20 years ago, our understanding of ATM, ATR, and DNA-PK has improved dramatically. Nevertheless, major questions still remain. One key issue is the current lack of high-resolution DDR-PIKK structures. Another is separating out functionally important phosphorylation events from the many residues that are modified simply because they by chance conform to DDR-PIKK consensus motifs. New and emerging technologies, such as super-resolution microscopy, cryo-electron microscopy, CRISPR-Cas9, and affordable high-throughput sequencing, will no doubt help address these and other issues. Taken together with the development of PIKK inhibitors, such advances will surely increase our understanding of PIKK functions in normal and diseased cells and thus hopefully pave the way for better treatments for cancer as well as other diseases associated with genome instability or aberrant PIKK function.

REFERENCES

Abraham, R.T. (1998). Mammalian target of rapamycin: immunosuppressive drugs uncover a novel pathway of cytokine receptor signaling. Curr. Opin. Immunol. 10, 330–336.

Adamowicz, M., Vermezovic, J., and d'Adda di Fagagna, F. (2016). NOTCH1 inhibits activation of ATM by impairing the formation of an ATM-FOXO3a-KAT5/Tip60 complex. Cell Rep. 16, 2068–2076.

Adams, K.E., Medhurst, A.L., Dart, D.A., and Lakin, N.D. (2006). Recruitment of ATR to sites of ionising radiation-induced DNA damage requires ATM and components of the MRN protein complex. Oncogene 25, 3894–3904.

Ahn, J.Y., Schwarz, J.K., Piwnica-Worms, H., and Canman, C.E. (2000). Threonine 68 phosphorylation by ataxia telangiectasia mutated is required for efficient activation of Chk2 in response to ionizing radiation. Cancer Res. 60, 5934–5936.

Ahnesorg, P., Smith, P., and Jackson, S.P. (2006). XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. Cell 124, 301–313.

al-Khodairy, F., and Carr, A.M. (1992). DNA repair mutants defining G2 checkpoint pathways in Schizosaccharomyces pombe. EMBO J. 11, 1343–1350.

Allen, J.B., Zhou, Z., Siede, W., Friedberg, E.C., and Elledge, S.J. (1994). The SAD1/RAD53 protein kinase controls multiple checkpoints and DNA damage-induced transcription in yeast. Genes Dev. 8, 2401–2415.

Alt, F.W., Zhang, Y., Meng, F.-L., Guo, C., and Schwer, B. (2013). Mechanisms of programmed DNA lesions and genomic instability in the immune system. Cell 152, 417–429.

Altmeyer, M., Neelsen, K.J., Teloni, F., Pozdnyakova, I., Pellegrino, S., Grøfte, M., Rask, M.-B.D., Streicher, W., Jungmichel, S., Nielsen, M.L., and Lukas, J. (2015). Liquid demixing of intrinsically disordered proteins is seeded by poly(ADP-ribose). Nat. Commun. 6, 8088.

Álvarez-Quilón, A., Serrano-Benítez, A., Lieberman, J.A., Quintero, C., Sánchez-Gutiérrez, D., Escudero, L.M., and Cortés-Ledesma, F. (2014). ATM specifically mediates repair of double-strand breaks with blocked DNA ends. Nat. Commun. 5, 3347.

Andegeko, Y., Moyal, L., Mittelman, L., Tsarfaty, I., Shiloh, Y., and Rotman, G. (2001). Nuclear retention of ATM at sites of DNA double strand breaks. J. Biol. Chem. 276, 38224–38230.

Andreassen, P.R., D'Andrea, A.D., and Taniguchi, T. (2004). ATR couples FANCD2 monoubiquitination to the DNA-damage response. Genes Dev. 18, 1958–1963.

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

Ball, H.L., Myers, J.S., and Cortez, D. (2005). ATRIP binding to replication protein A-single-stranded DNA promotes ATR-ATRIP localization but is dispensable for Chk1 phosphorylation. Mol. Biol. Cell 16, 2372–2381.

Balmus, G., Zhu, M., Mukherjee, S., Lyndaker, A.M., Hume, K.R., Lee, J., Riccio, M.L., Reeves, A.P., Sutter, N.B., Noden, D.M., et al. (2012). Disease severity in a mouse model of ataxia telangiectasia is modulated by the DNA damage checkpoint gene Hus1. Hum. Mol. Genet. 21, 3408–3420.

Balmus, G., Barros, A.C., Wijnhoven, P.W.G., Lescale, C., Hasse, H.L., Boroviak, K., le Sage, C., Doe, B., Speak, A.O., Galli, A., et al. (2016). Synthetic lethality between PAXX and XLF in mammalian development. Genes Dev. 30, 2152–2157.

Banin, S., Moyal, L., Shieh, S., Taya, Y., Anderson, C.W., Chessa, L., Smorodinsky, N.I., Prives, C., Reiss, Y., Shiloh, Y., and Ziv, Y. (1998). Enhanced phosphorylation of p53 by ATM in response to DNA damage. Science 281, 1674–1677.

Bannister, A.J., Gottlieb, T.M., Kouzarides, T., and Jackson, S.P. (1993). c-Jun is phosphorylated by the DNA-dependent protein kinase in vitro; definition of the minimal kinase recognition motif. Nucleic Acids Res. 21, 1289–1295.

Bartek, J., Lukas, C., and Lukas, J. (2004). Checking on DNA damage in S phase. Nat. Rev. Mol. Cell Biol. 5, 792–804.

Bartkova, J., Horejsí, Z., Koed, K., Krämer, A., Tort, F., Zieger, K., Guldberg, P., Sehested, M., Nesland, J.M., Lukas, C., et al. (2005). DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 434, 864–870.

Bartkova, J., Rezaei, N., Liontos, M., Karakaidos, P., Kletsas, D., Issaeva, N., Vassiliou, L.-V.F., Kolettas, E., Niforou, K., Zoumpourlis, V.C., et al. (2006). Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature 444, 633–637.

Baskaran, R., Wood, L.D., Whitaker, L.L., Canman, C.E., Morgan, S.E., Xu, Y., Barlow, C., Baltimore, D., Wynshaw-Boris, A., Kastan, M.B., and Wang, J.Y. (1997). Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation. Nature 387, 516–519.

Bass, T.E., Luzwick, J.W., Kavanaugh, G., Carroll, C., Dungrawala, H., Glick, G.G., Feldkamp, M.D., Putney, R., Chazin, W.J., and Cortez, D. (2016). ETAA1 acts at stalled replication forks to maintain genome integrity. Nat. Cell Biol. 18, 1185–1195.

Bekker-Jensen, S., Lukas, C., Kitagawa, R., Melander, F., Kastan, M.B., Bartek, J., and Lukas, J. (2006). Spatial organization of the mammalian genome surveillance machinery in response to DNA strand breaks. J. Cell Biol. 173, 195–206.

Bentley, N.J., Holtzman, D.A., Flaggs, G., Keegan, K.S., DeMaggio, A., Ford, J.C., Hoekstra, M., and Carr, A.M. (1996). The Schizosaccharomyces pombe rad3 checkpoint gene. EMBO J. *15*, 6641–6651.

Bermejo, R., Capra, T., Jossen, R., Colosio, A., Frattini, C., Carotenuto, W., Cocito, A., Doksani, Y., Klein, H., Gómez-González, B., et al. (2011). The replication checkpoint protects fork stability by releasing transcribed genes from nuclear pores. Cell *146*, 233–246.

Bétermier, M., Bertrand, P., and Lopez, B.S. (2014). Is non-homologous end-joining really an inherently error-prone process? PLoS Genet. *10*, e1004086.

Beucher, A., Birraux, J., Tchouandong, L., Barton, O., Shibata, A., Conrad, S., Goodarzi, A.A., Krempler, A., Jeggo, P.A., and Löbrich, M. (2009). ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G2. EMBO J. *28*, 3413–3427.

Blackford, A.N., Patel, R.N., Forrester, N.A., Theil, K., Groitl, P., Stewart, G.S., Taylor, A.M.R., Morgan, I.M., Dobner, T., Grand, R.J.A., and Turnell, A.S. (2010). Adenovirus 12 E4orf6 inhibits ATR activation by promoting TOPBP1 degradation. Proc. Natl. Acad. Sci. USA *107*, 12251–12256.

Blackford, A.N., Nieminuszczy, J., Schwab, R.A., Galanty, Y., Jackson, S.P., and Niedzwiedz, W. (2015). TopBP1 interacts with BLM to maintain genome stability but is dispensable for preventing BLM degradation. Mol. Cell *57*, 1133–1141.

Blunt, T., Finnie, N.J., Taccioli, G.E., Smith, G.C., Demengeot, J., Gottlieb, T.M., Mizuta, R., Varghese, A.J., Alt, F.W., Jeggo, P.A., and Jackson, S.P. (1995). Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine scid mutation. Cell *80*, 813–823.

Bosma, G.C., Custer, R.P., and Bosma, M.J. (1983). A severe combined immunodeficiency mutation in the mouse. Nature *301*, 527–530.

Bosotti, R., Isacchi, A., and Sonnhammer, E.L. (2000). FAT: a novel domain in PIK-related kinases. Trends Biochem. Sci. *25*, 225–227.

Britton, S., Coates, J., and Jackson, S.P. (2013). A new method for high-resolution imaging of Ku foci to decipher mechanisms of DNA double-strand break repair. J. Cell Biol. *202*, 579–595.

Britton, S., Dernoncourt, E., Delteil, C., Froment, C., Schiltz, O., Salles, B., Frit, P., and Calsou, P. (2014). DNA damage triggers SAF-A and RNA biogenesis factors exclusion from chromatin coupled to R-loops removal. Nucleic Acids Res. *42*, 9047–9062.

Brown, E.J., and Baltimore, D. (2000). ATR disruption leads to chromosomal fragmentation and early embryonic lethality. Genes Dev. *14*, 397–402.

Brown, A.L., Lee, C.H., Schwarz, J.K., Mitiku, N., Piwnica-Worms, H., and Chung, J.H. (1999). A human Cds1-related kinase that functions downstream of ATM protein in the cellular response to DNA damage. Proc. Natl. Acad. Sci. USA *96*, 3745–3750.

Brown, J.S., O'Carrigan, B., Jackson, S.P., and Yap, T.A. (2017). Targeting DNA repair in cancer: beyond PARP inhibitors. Cancer Discov. *7*, 20–37.

Bryant, H.E., Schultz, N., Thomas, H.D., Parker, K.M., Flower, D., Lopez, E., Kyle, S., Meuth, M., Curtin, N.J., and Helleday, T. (2005). Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature *434*, 913–917.

Buck, D., Malivert, L., de Chasseval, R., Barraud, A., Fondanèche, M.-C., Sanal, O., Plebani, A., Stéphan, J.-L., Hufnagel, M., le Deist, F., et al. (2006). Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. Cell *124*, 287–299.

Buisson, R., Boisvert, J.L., Benes, C.H., and Zou, L. (2015). Distinct but concerted roles of ATR, DNA-PK, and Chk1 in countering replication stress during S phase. Mol. Cell *59*, 1011–1024.

Byun, T.S., Pacek, M., Yee, M.-C., Walter, J.C., and Cimprich, K.A. (2005). Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. Genes Dev. *19*, 1040–1052.

Canman, C.E., Lim, D.S., Cimprich, K.A., Taya, Y., Tamai, K., Sakaguchi, K., Appella, E., Kastan, M.B., and Siliciano, J.D. (1998). Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. Science *281*, 1677–1679.

Carette, J.E., Guimaraes, C.P., Wuethrich, I., Blomen, V.A., Varadarajan, M., Sun, C., Bell, G., Yuan, B., Muellner, M.K., Nijman, S.M., et al. (2011). Global gene disruption in human cells to assign genes to phenotypes by deep sequencing. Nat. Biotechnol. *29*, 542–546.

Carney, J.P., Maser, R.S., Olivares, H., Davis, E.M., Le Beau, M., Yates, J.R., 3rd, Hays, L., Morgan, W.F., and Petrini, J.H. (1998). The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. Cell *93*, 477–486.

Caron, P., Choudjaye, J., Clouaire, T., Bugler, B., Daburon, V., Aguirrebengoa, M., Mangeat, T., Iacovoni, J.S., Álvarez-Quilón, A., Cortés-Ledesma, F., and Legube, G. (2015). Non-redundant functions of ATM and DNA-PKcs in response to DNA double-strand breaks. Cell Rep. *13*, 1598–1609.

Carson, C.T., Schwartz, R.A., Stracker, T.H., Lilley, C.E., Lee, D.V., and Weitzman, M.D. (2003). The Mre11 complex is required for ATM activation and the G2/M checkpoint. EMBO J. *22*, 6610–6620.

Carter, T., Vancurová, I., Sun, I., Lou, W., and DeLeon, S. (1990). A DNA-activated protein kinase from HeLa cell nuclei. Mol. Cell. Biol. *10*, 6460–6471.

Ceccaldi, R., Sarangi, P., and D'Andrea, A.D. (2016). The Fanconi anaemia pathway: new players and new functions. Nat. Rev. Mol. Cell Biol. *17*, 337–349.

Chan, D.W., Chen, B.P.-C., Prithivirajsingh, S., Kurimasa, A., Story, M.D., Qin, J., and Chen, D.J. (2002). Autophosphorylation of the DNA-dependent protein kinase catalytic subunit is required for rejoining of DNA double-strand breaks. Genes Dev. *16*, 2333–2338.

Chanut, P., Britton, S., Coates, J., Jackson, S.P., and Calsou, P. (2016). Coordinated nuclease activities counteract Ku at single-ended DNA double-strand breaks. Nat. Commun. *7*, 12889.

Chapman, J.R., and Jackson, S.P. (2008). Phospho-dependent interactions between NBS1 and MDC1 mediate chromatin retention of the MRN complex at sites of DNA damage. EMBO Rep. *9*, 795–801.

Chapman, J.R., Taylor, M.R.G., and Boulton, S.J. (2012). Playing the end game: DNA double-strand break repair pathway choice. Mol. Cell *47*, 497–510.

Chen, Y.R., Lees-Miller, S.P., Tegtmeyer, P., and Anderson, C.W. (1991). The human DNA-activated protein kinase phosphorylates simian virus 40 T antigen at amino- and carboxy-terminal sites. J. Virol. *65*, 5131–5140.

Chen, B.P.C., Chan, D.W., Kobayashi, J., Burma, S., Asaithamby, A., Morotomi-Yano, K., Botvinick, E., Qin, J., and Chen, D.J. (2005). Cell cycle dependence of DNA-dependent protein kinase phosphorylation in response to DNA double strand breaks. J. Biol. Chem. *280*, 14709–14715.

Chen, B.P.C., Uematsu, N., Kobayashi, J., Lerenthal, Y., Krempler, A., Yajima, H., Löbrich, M., Shiloh, Y., and Chen, D.J. (2007). Ataxia telangiectasia mutated (ATM) is essential for DNA-PKcs phosphorylations at the Thr-2609 cluster upon DNA double strand break. J. Biol. Chem. *282*, 6582–6587.

Chen, Y.-H., Jones, M.J.K., Yin, Y., Crist, S.B., Colnaghi, L., Sims, R.J., 3rd, Rothenberg, E., Jallepalli, P.V., and Huang, T.T. (2015). ATR-mediated phosphorylation of FANCI regulates dormant origin firing in response to replication stress. Mol. Cell *58*, 323–338.

Cheng, Q., and Chen, J. (2010). Mechanism of p53 stabilization by ATM after DNA damage. Cell Cycle *9*, 472–478.

Chou, D.M., Adamson, B., Dephoure, N.E., Tan, X., Nottke, A.C., Hurov, K.E., Gygi, S.P., Colaiácovo, M.P., and Elledge, S.J. (2010). A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. Proc. Natl. Acad. Sci. USA *107*, 18475–18480.

Ciccia, A., and Elledge, S.J. (2010). The DNA damage response: making it safe to play with knives. Mol. Cell *40*, 179–204.

Cimprich, K.A., Shin, T.B., Keith, C.T., and Schreiber, S.L. (1996). cDNA cloning and gene mapping of a candidate human cell cycle checkpoint protein. Proc. Natl. Acad. Sci. USA *93*, 2850–2855.

Cliby, W.A., Roberts, C.J., Cimprich, K.A., Stringer, C.M., Lamb, J.R., Schreiber, S.L., and Friend, S.H. (1998). Overexpression of a kinase-inactive ATR protein causes sensitivity to DNA-damaging agents and defects in cell cycle checkpoints. EMBO J. *17*, 159–169.

Cortez, D. (2015). Preventing replication fork collapse to maintain genome integrity. DNA Repair (Amst.) *32*, 149–157.

Cortez, D., Wang, Y., Qin, J., and Elledge, S.J. (1999). Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. Science *286*, 1162–1166.

Costanzo, V., Shechter, D., Lupardus, P.J., Cimprich, K.A., Gottesman, M., and Gautier, J. (2003). An ATR- and Cdc7-dependent DNA damage checkpoint that inhibits initiation of DNA replication. Mol. Cell *11*, 203–213.

Couch, F.B., Bansbach, C.E., Driscoll, R., Luzwick, J.W., Glick, G.G., Bétous, R., Carroll, C.M., Jung, S.Y., Qin, J., Cimprich, K.A., and Cortez, D. (2013). ATR phosphorylates SMARCAL1 to prevent replication fork collapse. Genes Dev. *27*, 1610–1623.

Critchlow, S.E., Bowater, R.P., and Jackson, S.P. (1997). Mammalian DNA double-strand break repair protein XRCC4 interacts with DNA ligase IV. Curr. Biol. *7*, 588–598.

Cuadrado, M., Martinez-Pastor, B., Murga, M., Toledo, L.I., Gutierrez-Martinez, P., Lopez, E., and Fernandez-Capetillo, O. (2006). ATM regulates ATR chromatin loading in response to DNA double-strand breaks. J. Exp. Med. *203*, 297–303.

d'Adda di Fagagna, F., Reaper, P.M., Clay-Farrace, L., Fiegler, H., Carr, P., Von Zglinicki, T., Saretzki, G., Carter, N.P., and Jackson, S.P. (2003). A DNA damage checkpoint response in telomere-initiated senescence. Nature *426*, 194–198.

D'Angiolella, V., Donato, V., Forrester, F.M., Jeong, Y.-T., Pellacani, C., Kudo, Y., Saraf, A., Florens, L., Washburn, M.P., and Pagano, M. (2012). Cyclin F-mediated degradation of ribonucleotide reductase M2 controls genome integrity and DNA repair. Cell *149*, 1023–1034.

Daniel, J.A., Pellegrini, M., Lee, J.-H., Paull, T.T., Feigenbaum, L., and Nussenzweig, A. (2008). Multiple autophosphorylation sites are dispensable for murine ATM activation in vivo. J. Cell Biol. *183*, 777–783.

de Klein, A., Muijtjens, M., van Os, R., Verhoeven, Y., Smit, B., Carr, A.M., Lehmann, A.R., and Hoeijmakers, J.H. (2000). Targeted disruption of the cell-cycle checkpoint gene ATR leads to early embryonic lethality in mice. Curr. Biol. *10*, 479–482.

de Lange, T. (2009). How telomeres solve the end-protection problem. Science *326*, 948–952.

De Piccoli, G., Katou, Y., Itoh, T., Nakato, R., Shirahige, K., and Labib, K. (2012). Replisome stability at defective DNA replication forks is independent of S phase checkpoint kinases. Mol. Cell *45*, 696–704.

Delacroix, S., Wagner, J.M., Kobayashi, M., Yamamoto, K., and Karnitz, L.M. (2007). The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1. Genes Dev. *21*, 1472–1477.

Douglas, P., Cui, X., Block, W.D., Yu, Y., Gupta, S., Ding, Q., Ye, R., Morrice, N., Lees-Miller, S.P., and Meek, K. (2007). The DNA-dependent protein kinase catalytic subunit is phosphorylated in vivo on threonine 3950, a highly conserved amino acid in the protein kinase domain. Mol. Cell. Biol. *27*, 1581–1591.

Dungrawala, H., Rose, K.L., Bhat, K.P., Mohni, K.N., Glick, G.G., Couch, F.B., and Cortez, D. (2015). The replication checkpoint prevents two types of fork collapse without regulating replisome stability. Mol. Cell *59*, 998–1010.

Dupré, A., Boyer-Chatenet, L., and Gautier, J. (2006). Two-step activation of ATM by DNA and the Mre11-Rad50-Nbs1 complex. Nat. Struct. Mol. Biol. *13*, 451–457.

Duursma, A.M., Driscoll, R., Elias, J.E., and Cimprich, K.A. (2013). A role for the MRN complex in ATR activation via TOPBP1 recruitment. Mol. Cell *50*, 116–122.

Dvir, A., Peterson, S.R., Knuth, M.W., Lu, H., and Dynan, W.S. (1992). Ku autoantigen is the regulatory component of a template-associated protein kinase that phosphorylates RNA polymerase II. Proc. Natl. Acad. Sci. USA *89*, 11920–11924.

Ellison, V., and Stillman, B. (2003). Biochemical characterization of DNA damage checkpoint complexes: clamp loader and clamp complexes with specificity for 5′ recessed DNA. PLoS Biol. *1*, E33.

Falck, J., Coates, J., and Jackson, S.P. (2005). Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. Nature *434*, 605–611.

Farmer, H., McCabe, N., Lord, C.J., Tutt, A.N.J., Johnson, D.A., Richardson, T.B., Santarosa, M., Dillon, K.J., Hickson, I., Knights, C., et al. (2005). Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature *434*, 917–921.

Feng, S., Zhao, Y., Xu, Y., Ning, S., Huo, W., Hou, M., Gao, G., Ji, J., Guo, R., and Xu, D. (2016). Ewing tumor-associated antigen 1 interacts with replication protein A to promote restart of stalled replication forks. J. Biol. Chem. *291*, 21956–21962.

Finnie, N.J., Gottlieb, T.M., Blunt, T., Jeggo, P.A., and Jackson, S.P. (1995). DNA-dependent protein kinase activity is absent in xrs-6 cells: implications for site-specific recombination and DNA double-strand break repair. Proc. Natl. Acad. Sci. USA *92*, 320–324.

Fokas, E., Prevo, R., Hammond, E.M., Brunner, T.B., McKenna, W.G., and Muschel, R.J. (2014). Targeting ATR in DNA damage response and cancer therapeutics. Cancer Treat. Rev. *40*, 109–117.

Forment, J.V., Blasius, M., Guerini, I., and Jackson, S.P. (2011). Structure-specific DNA endonuclease Mus81/Eme1 generates DNA damage caused by Chk1 inactivation. PLoS ONE *6*, e23517.

Fradet-Turcotte, A., Canny, M.D., Escribano-Díaz, C., Orthwein, A., Leung, C.C.Y., Huang, H., Landry, M.-C., Kitevski-LeBlanc, J., Noordermeer, S.M., Sicheri, F., and Durocher, D. (2013). 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. Nature *499*, 50–54.

Gao, Y., Sun, Y., Frank, K.M., Dikkes, P., Fujiwara, Y., Seidl, K.J., Sekiguchi, J.M., Rathbun, G.A., Swat, W., Wang, J., et al. (1998). A critical role for DNA end-joining proteins in both lymphogenesis and neurogenesis. Cell *95*, 891–902.

Gell, D., and Jackson, S.P. (1999). Mapping of protein-protein interactions within the DNA-dependent protein kinase complex. Nucleic Acids Res. *27*, 3494–3502.

Getts, R.C., and Stamato, T.D. (1994). Absence of a Ku-like DNA end binding activity in the xrs double-strand DNA repair-deficient mutant. J. Biol. Chem. *269*, 15981–15984.

Goodarzi, A.A., Yu, Y., Riballo, E., Douglas, P., Walker, S.A., Ye, R., Härer, C., Marchetti, C., Morrice, N., Jeggo, P.A., and Lees-Miller, S.P. (2006). DNA-PK autophosphorylation facilitates Artemis endonuclease activity. EMBO J. *25*, 3880–3889.

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

Gorgoulis, V.G., Vassiliou, L.-V.F., Karakaidos, P., Zacharatos, P., Kotsinas, A., Liloglou, T., Venere, M., Ditullio, R.A., Jr., Kastrinakis, N.G., Levy, B., et al. (2005). Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature *434*, 907–913.

Gotoff, S.P., Amirmokri, E., and Liebner, E.J. (1967). Ataxia telangiectasia. Neoplasia, untoward response to x-irradiation, and tuberous sclerosis. Am. J. Dis. Child. *114*, 617–625.

Gottlieb, T.M., and Jackson, S.P. (1993). The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell *72*, 131–142.

Graham, T.G.W., Walter, J.C., and Loparo, J.J. (2016). Two-stage synapsis of DNA ends during non-homologous end joining. Mol. Cell *61*, 850–858.

Grawunder, U., Wilm, M., Wu, X., Kulesza, P., Wilson, T.E., Mann, M., and Lieber, M.R. (1997). Activity of DNA ligase IV stimulated by complex formation with XRCC4 protein in mammalian cells. Nature *388*, 492–495.

Greenwell, P.W., Kronmal, S.L., Porter, S.E., Gassenhuber, J., Obermaier, B., and Petes, T.D. (1995). TEL1, a gene involved in controlling telomere length in *S. cerevisiae*, is homologous to the human ataxia telangiectasia gene. Cell *82*, 823–829.

Grinthal, A., Adamovic, I., Weiner, B., Karplus, M., and Kleckner, N. (2010). PR65, the HEAT-repeat scaffold of phosphatase PP2A, is an elastic connector that links force and catalysis. Proc. Natl. Acad. Sci. USA *107*, 2467–2472.

Guo, Z., Kumagai, A., Wang, S.X., and Dunphy, W.G. (2000). Requirement for Atr in phosphorylation of Chk1 and cell cycle regulation in response to DNA replication blocks and UV-damaged DNA in Xenopus egg extracts. Genes Dev. 14, 2745–2756.

Guo, Z., Kozlov, S., Lavin, M.F., Person, M.D., and Paull, T.T. (2010). ATM activation by oxidative stress. Science 330, 517–521.

Gurley, K.E., and Kemp, C.J. (2001). Synthetic lethality between mutation in Atm and DNA-PK(cs) during murine embryogenesis. Curr. Biol. 11, 191–194.

Haahr, P., Hoffmann, S., Tollenaere, M.A.X., Ho, T., Toledo, L.I., Mann, M., Bekker-Jensen, S., Räschle, M., and Mailand, N. (2016). Activation of the ATR kinase by the RPA-binding protein ETAA1. Nat. Cell Biol. 18, 1196–1207.

Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. Cell 144, 646–674.

Harrigan, J.A., Belotserkovskaya, R., Coates, J., Dimitrova, D.S., Polo, S.E., Bradshaw, C.R., Fraser, P., and Jackson, S.P. (2011). Replication stress induces 53BP1-containing OPT domains in G1 cells. J. Cell Biol. 193, 97–108.

Hartley, K.O., Gell, D., Smith, G.C., Zhang, H., Divecha, N., Connelly, M.A., Admon, A., Lees-Miller, S.P., Anderson, C.W., and Jackson, S.P. (1995). DNA-dependent protein kinase catalytic subunit: a relative of phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product. Cell 82, 849–856.

Hekmat-Nejad, M., You, Z., Yee, M.C., Newport, J.W., and Cimprich, K.A. (2000). Xenopus ATR is a replication-dependent chromatin-binding protein required for the DNA replication checkpoint. Curr. Biol. 10, 1565–1573.

Hirao, A., Cheung, A., Duncan, G., Girard, P.-M., Elia, A.J., Wakeham, A., Okada, H., Sarkissian, T., Wong, J.A., Sakai, T., et al. (2002). Chk2 is a tumor suppressor that regulates apoptosis in both an ataxia telangiectasia mutated (ATM)-dependent and an ATM-independent manner. Mol. Cell. Biol. 22, 6521–6532.

Houldsworth, J., and Lavin, M.F. (1980). Effect of ionizing radiation on DNA synthesis in ataxia telangiectasia cells. Nucleic Acids Res. 8, 3709–3720.

Hsu, P.D., Lander, E.S., and Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. Cell 157, 1262–1278.

Huen, M.S.Y., Grant, R., Manke, I., Minn, K., Yu, X., Yaffe, M.B., and Chen, J. (2007). RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. Cell 131, 901–914.

Hustedt, N., and Durocher, D. (2016). The control of DNA repair by the cell cycle. Nat. Cell Biol. 19, 1–9.

Imray, F.P., and Kidson, C. (1983). Perturbations of cell-cycle progression in gamma-irradiated ataxia telangiectasia and Huntington's disease cells detected by DNA flow cytometric analysis. Mutat. Res. 112, 369–382.

Ishiai, M., Kitao, H., Smogorzewska, A., Tomida, J., Kinomura, A., Uchida, E., Saberi, A., Kinoshita, E., Kinoshita-Kikuta, E., Koike, T., et al. (2008). FANCI phosphorylation functions as a molecular switch to turn on the Fanconi anemia pathway. Nat. Struct. Mol. Biol. 15, 1138–1146.

Jack, M.T., Woo, R.A., Hirao, A., Cheung, A., Mak, T.W., and Lee, P.W.K. (2002). Chk2 is dispensable for p53-mediated G1 arrest but is required for a latent p53-mediated apoptotic response. Proc. Natl. Acad. Sci. USA 99, 9825–9829.

Jackson, S.P., and Bartek, J. (2009). The DNA-damage response in human biology and disease. Nature 461, 1071–1078.

Jackson, S.P., MacDonald, J.J., Lees-Miller, S., and Tjian, R. (1990). GC box binding induces phosphorylation of Sp1 by a DNA-dependent protein kinase. Cell 63, 155–165.

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

Jeggo, P.A., Taccioli, G.E., and Jackson, S.P. (1995). Menage à trois: double strand break repair, V(D)J recombination and DNA-PK. BioEssays 17, 949–957.

Jeon, Y., Ko, E., Lee, K.Y., Ko, M.J., Park, S.Y., Kang, J., Jeon, C.H., Lee, H., and Hwang, D.S. (2011). TopBP1 deficiency causes an early embryonic lethality and induces cellular senescence in primary cells. J. Biol. Chem. 286, 5414–5422.

Jette, N., and Lees-Miller, S.P. (2015). The DNA-dependent protein kinase: A multifunctional protein kinase with roles in DNA double strand break repair and mitosis. Prog. Biophys. Mol. Biol. 117, 194–205.

Jiang, W., Crowe, J.L., Liu, X., Nakajima, S., Wang, Y., Li, C., Lee, B.J., Dubois, R.L., Liu, C., Yu, X., et al. (2015). Differential phosphorylation of DNA-PKcs regulates the interplay between end-processing and end-ligation during nonhomologous end-joining. Mol. Cell 58, 172–185.

Jimenez, G., Yucel, J., Rowley, R., and Subramani, S. (1992). The rad3+ gene of Schizosaccharomyces pombe is involved in multiple checkpoint functions and in DNA repair. Proc. Natl. Acad. Sci. USA 89, 4952–4956.

Jowsey, P.A., Doherty, A.J., and Rouse, J. (2004). Human PTIP facilitates ATM-mediated activation of p53 and promotes cellular resistance to ionizing radiation. J. Biol. Chem. 279, 55562–55569.

Jungmichel, S., Clapperton, J.A., Lloyd, J., Hari, F.J., Spycher, C., Pavic, L., Li, J., Haire, L.F., Bonalli, M., Larsen, D.H., et al. (2012). The molecular basis of ATM-dependent dimerization of the Mdc1 DNA damage checkpoint mediator. Nucleic Acids Res. 40, 3913–3928.

Kaidi, A., and Jackson, S.P. (2013). KAT5 tyrosine phosphorylation couples chromatin sensing to ATM signalling. Nature 498, 70–74.

Kakarougkas, A., Ismail, A., Chambers, A.L., Riballo, E., Herbert, A.D., Künzel, J., Löbrich, M., Jeggo, P.A., and Downs, J.A. (2014). Requirement for PBAF in transcriptional repression and repair at DNA breaks in actively transcribed regions of chromatin. Mol. Cell 55, 723–732.

Karanam, K., Kafri, R., Loewer, A., and Lahav, G. (2012). Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase. Mol. Cell 47, 320–329.

Kastan, M.B., Zhan, Q., el-Deiry, W.S., Carrier, F., Jacks, T., Walsh, W.V., Plunkett, B.S., Vogelstein, B., and Fornace, A.J., Jr. (1992). A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell 71, 587–597.

Kato, R., and Ogawa, H. (1994). An essential gene, ESR1, is required for mitotic cell growth, DNA repair and meiotic recombination in Saccharomyces cerevisiae. Nucleic Acids Res. 22, 3104–3112.

Kim, S.T., Lim, D.S., Canman, C.E., and Kastan, M.B. (1999). Substrate specificities and identification of putative substrates of ATM kinase family members. J. Biol. Chem. 274, 37538–37543.

Kirchgessner, C.U., Patil, C.K., Evans, J.W., Cuomo, C.A., Fried, L.M., Carter, T., Oettinger, M.A., and Brown, J.M. (1995). DNA-dependent kinase (p350) as a candidate gene for the murine SCID defect. Science 267, 1178–1183.

Kolas, N.K., Chapman, J.R., Nakada, S., Ylanko, J., Chahwan, R., Sweeney, F.D., Panier, S., Mendez, M., Wildenhain, J., Thomson, T.M., et al. (2007). Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. Science 318, 1637–1640.

Kowalczykowski, S.C. (2015). An overview of the molecular mechanisms of recombinational DNA repair. Cold Spring Harb. Perspect. Biol. 7, http://dx.doi.org/10.1101/cshperspect.a016410.

Kozlov, S.V., Graham, M.E., Peng, C., Chen, P., Robinson, P.J., and Lavin, M.F. (2006). Involvement of novel autophosphorylation sites in ATM activation. EMBO J. 25, 3504–3514.

Kruhlak, M., Crouch, E.E., Orlov, M., Montaño, C., Gorski, S.A., Nussenzweig, A., Misteli, T., Phair, R.D., and Casellas, R. (2007). The ATM repair pathway inhibits RNA polymerase I transcription in response to chromosome breaks. Nature 447, 730–734.

Kumagai, A., Lee, J., Yoo, H.Y., and Dunphy, W.G. (2006). TopBP1 activates the ATR-ATRIP complex. Cell 124, 943–955.

Kumar, A., Mazzanti, M., Mistrik, M., Kosar, M., Beznoussenko, G.V., Mironov, A.A., Garrè, M., Parazzoli, D., Shivashankar, G.V., Scita, G., et al. (2014a). ATR mediates a checkpoint at the nuclear envelope in response to mechanical stress. Cell 158, 633–646.

Kumar, V., Alt, F.W., and Oksenych, V. (2014b). Functional overlaps between XLF and the ATM-dependent DNA double strand break response. DNA Repair (Amst.) 16, 11–22.

Kumar, V., Alt, F.W., and Frock, R.L. (2016). PAXX and XLF DNA repair factors are functionally redundant in joining DNA breaks in a G1-arrested progenitor B-cell line. Proc. Natl. Acad. Sci. USA 113, 10619–10624.

Lakin, N.D., Hann, B.C., and Jackson, S.P. (1999). The ataxia-telangiectasia related protein ATR mediates DNA-dependent phosphorylation of p53. Oncogene 18, 3989–3995.

Lau, A., Swinbank, K.M., Ahmed, P.S., Taylor, D.L., Jackson, S.P., Smith, G.C.M., and O'Connor, M.J. (2005). Suppression of HIV-1 infection by a small molecule inhibitor of the ATM kinase. Nat. Cell Biol. 7, 493–500.

Lau, W.C.Y., Li, Y., Liu, Z., Gao, Y., Zhang, Q., and Huen, M.S.Y. (2016). Structure of the human dimeric ATM kinase. Cell Cycle 15, 1117–1124.

Lecona, E., and Fernández-Capetillo, O. (2014). Replication stress and cancer: it takes two to tango. Exp. Cell Res. 329, 26–34.

Lee, J., and Dunphy, W.G. (2010). Rad17 plays a central role in establishment of the interaction between TopBP1 and the Rad9-Hus1-Rad1 complex at stalled replication forks. Mol. Biol. Cell 21, 926–935.

Lee, J.-H., and Paull, T.T. (2004). Direct activation of the ATM protein kinase by the Mre11/Rad50/Nbs1 complex. Science 304, 93–96.

Lee, J.-H., and Paull, T.T. (2005). ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. Science 308, 551–554.

Lee, J., Kumagai, A., and Dunphy, W.G. (2007). The Rad9-Hus1-Rad1 checkpoint clamp regulates interaction of TopBP1 with ATR. J. Biol. Chem. 282, 28036–28044.

Lees-Miller, S.P., and Anderson, C.W. (1989). The human double-stranded DNA-activated protein kinase phosphorylates the 90-kDa heat-shock protein, hsp90 alpha at two NH2-terminal threonine residues. J. Biol. Chem. 264, 17275–17280.

Lees-Miller, S.P., Chen, Y.R., and Anderson, C.W. (1990). Human cells contain a DNA-activated protein kinase that phosphorylates simian virus 40 T antigen, mouse p53, and the human Ku autoantigen. Mol. Cell. Biol. 10, 6472–6481.

Lees-Miller, S.P., Godbout, R., Chan, D.W., Weinfeld, M., Day, R.S., 3rd, Barron, G.M., and Allalunis-Turner, J. (1995). Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line. Science 267, 1183–1185.

Lees-Miller, S.P., Long, M.C., Kilvert, M.A., Lam, V., Rice, S.A., and Spencer, C.A. (1996). Attenuation of DNA-dependent protein kinase activity and its catalytic subunit by the herpes simplex virus type 1 transactivator ICP0. J. Virol. 70, 7471–7477.

Lescale, C., Lenden Hasse, H., Blackford, A.N., Balmus, G., Bianchi, J.J., Yu, W., Bacoccina, L., Jarade, A., Clouin, C., Sivapalan, R., et al. (2016). Specific roles of XRCC4 paralogs PAXX and XLF during V(D)J recombination. Cell Rep. 16, 2967–2979.

Li, Z., Otevrel, T., Gao, Y., Cheng, H.L., Seed, B., Stamato, T.D., Taccioli, G.E., and Alt, F.W. (1995). The XRCC4 gene encodes a novel protein involved in DNA double-strand break repair and V(D)J recombination. Cell 83, 1079–1089.

Liu, Q., Guntuku, S., Cui, X.S., Matsuoka, S., Cortez, D., Tamai, K., Luo, G., Carattini-Rivera, S., DeMayo, F., Bradley, A., et al. (2000). Chk1 is an essential kinase that is regulated by Atr and required for the G(2)/M DNA damage checkpoint. Genes Dev. 14, 1448–1459.

Liu, S., Shiotani, B., Lahiri, M., Maréchal, A., Tse, A., Leung, C.C.Y., Glover, J.N.M., Yang, X.H., and Zou, L. (2011). ATR autophosphorylation as a molecular switch for checkpoint activation. Mol. Cell 43, 192–202.

Liu, J., Luo, S., Zhao, H., Liao, J., Li, J., Yang, C., Xu, B., Stern, D.F., Xu, X., and Ye, K. (2012). Structural mechanism of the phosphorylation-dependent dimerization of the MDC1 forkhead-associated domain. Nucleic Acids Res. 40, 3898–3912.

Liu, X., Shao, Z., Jiang, W., Lee, B.J., and Zha, S. (2017). PAXX promotes KU accumulation at DNA breaks and is essential for end-joining in XLF-deficient mice. Nat. Commun. 8, 13816.

López-Contreras, A.J., Specks, J., Barlow, J.H., Ambrogio, C., Desler, C., Vikingsson, S., Rodrigo-Perez, S., Green, H., Rasmussen, L.J., Murga, M., et al. (2015). Increased Rrm2 gene dosage reduces fragile site breakage and prolongs survival of ATR mutant mice. Genes Dev. 29, 690–695.

Lord, C.J., and Ashworth, A. (2017). PARP inhibitors: synthetic lethality in the clinic. Science 355, 1152–1158.

Lu, X.-H., Mattis, V.B., Wang, N., Al-Ramahi, I., van den Berg, N., Fratantoni, S.A., Waldvogel, H., Greiner, E., Osmand, A., Elzein, K., et al. (2014). Targeting ATM ameliorates mutant Huntingtin toxicity in cell and animal models of Huntington's disease. Sci. Transl. Med. 6, 268ra178.

Lukas, C., Savic, V., Bekker-Jensen, S., Doil, C., Neumann, B., Pedersen, R.S., Grøfte, M., Chan, K.L., Hickson, I.D., Bartek, J., and Lukas, J. (2011). 53BP1 nuclear bodies form around DNA lesions generated by mitotic transmission of chromosomes under replication stress. Nat. Cell Biol. 13, 243–253.

Lustig, A.J., and Petes, T.D. (1986). Identification of yeast mutants with altered telomere structure. Proc. Natl. Acad. Sci. USA 83, 1398–1402.

Ma, Y., Pannicke, U., Schwarz, K., and Lieber, M.R. (2002). Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. Cell 108, 781–794.

Macheret, M., and Halazonetis, T.D. (2015). DNA replication stress as a hallmark of cancer. Annu. Rev. Pathol. 10, 425–448.

Mailand, N., Bekker-Jensen, S., Faustrup, H., Melander, F., Bartek, J., Lukas, C., and Lukas, J. (2007). RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. Cell 131, 887–900.

Malu, S., De Ioannes, P., Kozlov, M., Greene, M., Francis, D., Hanna, M., Pena, J., Escalante, C.R., Kurosawa, A., Erdjument-Bromage, H., et al. (2012). Artemis C-terminal region facilitates V(D)J recombination through its interactions with DNA Ligase IV and DNA-PKcs. J. Exp. Med. 209, 955–963.

Matsuoka, S., Huang, M., and Elledge, S.J. (1998). Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. Science 282, 1893–1897.

Matsuoka, S., Rotman, G., Ogawa, A., Shiloh, Y., Tamai, K., and Elledge, S.J. (2000). Ataxia telangiectasia-mutated phosphorylates Chk2 in vivo and in vitro. Proc. Natl. Acad. Sci. USA 97, 10389–10394.

Matsuoka, S., Ballif, B.A., Smogorzewska, A., McDonald, E.R., 3rd, Hurov, K.E., Luo, J., Bakalarski, C.E., Zhao, Z., Solimini, N., Lerenthal, Y., et al. (2007). ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science 316, 1160–1166.

Mattiroli, F., Vissers, J.H.A., van Dijk, W.J., Ikpa, P., Citterio, E., Vermeulen, W., Marteijn, J.A., and Sixma, T.K. (2012). RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. Cell 150, 1182–1195.

Meek, K., Douglas, P., Cui, X., Ding, Q., and Lees-Miller, S.P. (2007). trans Autophosphorylation at DNA-dependent protein kinase's two major autophosphorylation site clusters facilitates end processing but not end joining. Mol. Cell. Biol. 27, 3881–3890.

Melander, F., Bekker-Jensen, S., Falck, J., Bartek, J., Mailand, N., and Lukas, J. (2008). Phosphorylation of SDT repeats in the MDC1 N terminus triggers retention of NBS1 at the DNA damage-modified chromatin. J. Cell Biol. 181, 213–226.

Melchionna, R., Chen, X.B., Blasina, A., and McGowan, C.H. (2000). Threonine 68 is required for radiation-induced phosphorylation and activation of Cds1. Nat. Cell Biol. 2, 762–765.

Middleton, F.K., Patterson, M.J., Elstob, C.J., Fordham, S., Herriott, A., Wade, M.A., McCormick, A., Edmondson, R., May, F.E.B., Allan, J.M., et al. (2015). Common cancer-associated imbalances in the DNA damage response confer sensitivity to single agent ATR inhibition. Oncotarget 6, 32396–32409.

Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P.A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L.M., Ding, W., et al. (1994). A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266, 66–71.

Mimori, T., and Hardin, J.A. (1986). Mechanism of interaction between Ku protein and DNA. J. Biol. Chem. 261, 10375–10379.

Mimori, T., Akizuki, M., Yamagata, H., Inada, S., Yoshida, S., and Homma, M. (1981). Characterization of a high molecular weight acidic nuclear protein recognized by autoantibodies in sera from patients with polymyositis-scleroderma overlap. J. Clin. Invest. 68, 611–620.

Mordes, D.A., Glick, G.G., Zhao, R., and Cortez, D. (2008). TopBP1 activates ATR through ATRIP and a PIKK regulatory domain. Genes Dev. 22, 1478–1489.

Moreira, M.-C., Klur, S., Watanabe, M., Németh, A.H., Le Ber, I., Moniz, J.-C., Tranchant, C., Aubourg, P., Tazir, M., Schöls, L., et al. (2004). Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. Nat. Genet. 36, 225–227.

Morrow, D.M., Tagle, D.A., Shiloh, Y., Collins, F.S., and Hieter, P. (1995). TEL1, an S. cerevisiae homolog of the human gene mutated in ataxia telangiectasia, is functionally related to the yeast checkpoint gene MEC1. Cell 82, 831–840.

Moshous, D., Callebaut, I., de Chasseval, R., Corneo, B., Cavazzana-Calvo, M., Le Deist, F., Tezcan, I., Sanal, O., Bertrand, Y., Philippe, N., et al. (2001). Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. Cell 105, 177–186.

Moumen, A., Masterson, P., O'Connor, M.J., and Jackson, S.P. (2005). hnRNP K: an HDM2 target and transcriptional coactivator of p53 in response to DNA damage. Cell 123, 1065–1078.

Moumen, A., Magill, C., Dry, K.L., and Jackson, S.P. (2013). ATM-dependent phosphorylation of heterogeneous nuclear ribonucleoprotein K promotes p53 transcriptional activation in response to DNA damage. Cell Cycle 12, 698–704.

Muñoz, I.M., Jowsey, P.A., Toth, R., and Rouse, J. (2007). Phospho-epitope binding by the BRCT domains of hPTIP controls multiple aspects of the cellular response to DNA damage. Nucleic Acids Res. 35, 5312–5322.

Myers, J.S., and Cortez, D. (2006). Rapid activation of ATR by ionizing radiation requires ATM and Mre11. J. Biol. Chem. 281, 9346–9350.

Nagasawa, H., and Little, J.B. (1983). Comparison of kinetics of X-ray-induced cell killing in normal, ataxia telangiectasia and hereditary retinoblastoma fibroblasts. Mutat. Res. 109, 297–308.

Nam, E.A., Zhao, R., Glick, G.G., Bansbach, C.E., Friedman, D.B., and Cortez, D. (2011). Thr-1989 phosphorylation is a marker of active ataxia telangiectasia-mutated and Rad3-related (ATR) kinase. J. Biol. Chem. 286, 28707–28714.

Nasim, A., and Smith, B.P. (1975). Genetic control of radiation sensitivity in Schizosaccharomyces pombe. Genetics 79, 573–582.

O'Connor, M.J. (2015). Targeting the DNA damage response in cancer. Mol. Cell 60, 547–560.

O'Driscoll, M., Ruiz-Perez, V.L., Woods, C.G., Jeggo, P.A., and Goodship, J.A. (2003). A splicing mutation affecting expression of ataxia-telangiectasia and Rad3-related protein (ATR) results in Seckel syndrome. Nat. Genet. 33, 497–501.

Ochi, T., Blackford, A.N., Coates, J., Jhujh, S., Mehmood, S., Tamura, N., Travers, J., Wu, Q., Draviam, V.M., Robinson, C.V., et al. (2015). DNA repair. PAXX, a paralog of XRCC4 and XLF, interacts with Ku to promote DNA double-strand break repair. Science 347, 185–188.

Ochs, F., Somyajit, K., Altmeyer, M., Rask, M.-B., Lukas, J., and Lukas, C. (2016). 53BP1 fosters fidelity of homology-directed DNA repair. Nat. Struct. Mol. Biol. 23, 714–721.

Olcina, M.M., Foskolou, I.P., Anbalagan, S., Senra, J.M., Pires, I.M., Jiang, Y., Ryan, A.J., and Hammond, E.M. (2013). Replication stress and chromatin context link ATM activation to a role in DNA replication. Mol. Cell 52, 758–766.

Orthwein, A., Noordermeer, S.M., Wilson, M.D., Landry, S., Enchev, R.I., Sherker, A., Munro, M., Pinder, J., Salsman, J., Dellaire, G., et al. (2015). A mechanism for the suppression of homologous recombination in G1 cells. Nature 528, 422–426.

Painter, R.B., and Young, B.R. (1980). Radiosensitivity in ataxia-telangiectasia: a new explanation. Proc. Natl. Acad. Sci. USA 77, 7315–7317.

Pankotai, T., Bonhomme, C., Chen, D., and Soutoglou, E. (2012). DNAPKcs-dependent arrest of RNA polymerase II transcription in the presence of DNA breaks. Nat. Struct. Mol. Biol. 19, 276–282.

Paulsen, R.D., Soni, D.V., Wollman, R., Hahn, A.T., Yee, M.-C., Guan, A., Hesley, J.A., Miller, S.C., Cromwell, E.F., Solow-Cordero, D.E., et al. (2009). A genome-wide siRNA screen reveals diverse cellular processes and pathways that mediate genome stability. Mol. Cell 35, 228–239.

Pellegrini, M., Celeste, A., Difilippantonio, S., Guo, R., Wang, W., Feigenbaum, L., and Nussenzweig, A. (2006). Autophosphorylation at serine 1987 is dispensable for murine Atm activation in vivo. Nature 443, 222–225.

Perry, J., and Kleckner, N. (2003). The ATRs, ATMs, and TORs are giant HEAT repeat proteins. Cell 112, 151–155.

Peterson, S.R., Kurimasa, A., Oshimura, M., Dynan, W.S., Bradbury, E.M., and Chen, D.J. (1995). Loss of the catalytic subunit of the DNA-dependent protein kinase in DNA double-strand-break-repair mutant mammalian cells. Proc. Natl. Acad. Sci. USA 92, 3171–3174.

Pfister, S.X., Markkanen, E., Jiang, Y., Sarkar, S., Woodcock, M., Orlando, G., Mavrommati, I., Pai, C.-C., Zalmas, L.-P., Drobnitzky, N., et al. (2015). Inhibiting WEE1 selectively kills histone H3K36me3-deficient cancers by dNTP starvation. Cancer Cell 28, 557–568.

Polo, S.E., Blackford, A.N., Chapman, J.R., Baskcomb, L., Gravel, S., Rusch, A., Thomas, A., Blundred, R., Smith, P., Kzhyshkowska, J., et al. (2012). Regulation of DNA-end resection by hnRNPU-like proteins promotes DNA double-strand break signaling and repair. Mol. Cell 45, 505–516.

Raderschall, E., Golub, E.I., and Haaf, T. (1999). Nuclear foci of mammalian recombination proteins are located at single-stranded DNA regions formed after DNA damage. Proc. Natl. Acad. Sci. USA 96, 1921–1926.

Ragland, R.L., Patel, S., Rivard, R.S., Smith, K., Peters, A.A., Bielinsky, A.-K., and Brown, E.J. (2013). RNF4 and PLK1 are required for replication fork collapse in ATR-deficient cells. Genes Dev. 27, 2259–2273.

Rathmell, W.K., and Chu, G. (1994). Involvement of the Ku autoantigen in the cellular response to DNA double-strand breaks. Proc. Natl. Acad. Sci. USA 91, 7623–7627.

Reaper, P.M., Griffiths, M.R., Long, J.M., Charrier, J.-D., Maccormick, S., Charlton, P.A., Golec, J.M.C., and Pollard, J.R. (2011). Selective killing of ATM- or p53-deficient cancer cells through inhibition of ATR. Nat. Chem. Biol. 7, 428–430.

Reeves, W.H., and Sthoeger, Z.M. (1989). Molecular cloning of cDNA encoding the p70 (Ku) lupus autoantigen. J. Biol. Chem. 264, 5047–5052.

Riballo, E., Kühne, M., Rief, N., Doherty, A., Smith, G.C.M., Recio, M.-J., Reis, C., Dahm, K., Fricke, A., Krempler, A., et al. (2004). A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci. Mol. Cell 16, 715–724.

Rogakou, E.P., Pilch, D.R., Orr, A.H., Ivanova, V.S., and Bonner, W.M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J. Biol. Chem. 273, 5858–5868.

Rooney, S., Alt, F.W., Lombard, D., Whitlow, S., Eckersdorff, M., Fleming, J., Fugmann, S., Ferguson, D.O., Schatz, D.G., and Sekiguchi, J. (2003). Defective DNA repair and increased genomic instability in Artemis-deficient murine cells. J. Exp. Med. 197, 553–565.

Rowley, R., Subramani, S., and Young, P.G. (1992). Checkpoint controls in Schizosaccharomyces pombe: rad1. EMBO J. 11, 1335–1342.

Royo, H., Prosser, H., Ruzankina, Y., Mahadevaiah, S.K., Cloutier, J.M., Baumann, M., Fukuda, T., Höög, C., Tóth, A., de Rooij, D.G., et al. (2013). ATR acts stage specifically to regulate multiple aspects of mammalian meiotic silencing. Genes Dev. 27, 1484–1494.

Ruiz, S., Mayor-Ruiz, C., Lafarga, V., Murga, M., Vega-Sendino, M., Ortega, S., and Fernandez-Capetillo, O. (2016). A genome-wide CRISPR screen identifies CDC25A as a determinant of sensitivity to ATR Inhibitors. Mol. Cell 62, 307–313.

Saito, S., Kurosawa, A., and Adachi, N. (2016). Mutations in XRCC4 cause primordial dwarfism without causing immunodeficiency. J. Hum. Genet. 61, 679–685.

Sartori, A.A., Lukas, C., Coates, J., Mistrik, M., Fu, S., Bartek, J., Baer, R., Lukas, J., and Jackson, S.P. (2007). Human CtIP promotes DNA end resection. Nature 450, 509–514.

Savitsky, K., Bar-Shira, A., Gilad, S., Rotman, G., Ziv, Y., Vanagaite, L., Tagle, D.A., Smith, S., Uziel, T., Sfez, S., et al. (1995). A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 268, 1749–1753.

Sawicka, M., Wanrooij, P.H., Darbari, V.C., Tannous, E., Hailemariam, S., Bose, D., Makarova, A.V., Burgers, P.M., and Zhang, X. (2016). The dimeric architecture of checkpoint kinases Mec1ATR and Tel1ATM reveal a common structural organization. J. Biol. Chem. 291, 13436–13447.

Scully, R., and Xie, A. (2013). Double strand break repair functions of histone H2AX. Mutat. Res. 750, 5–14.

Seaton, B.L., Yucel, J., Sunnerhagen, P., and Subramani, S. (1992). Isolation and characterization of the Schizosaccharomyces pombe rad3 gene, involved in the DNA damage and DNA synthesis checkpoints. Gene 119, 83–89.

Sekiguchi, J., Ferguson, D.O., Chen, H.T., Yang, E.M., Earle, J., Frank, K., Whitlow, S., Gu, Y., Xu, Y., Nussenzweig, A., and Alt, F.W. (2001). Genetic interactions between ATM and the nonhomologous end-joining factors in genomic stability and development. Proc. Natl. Acad. Sci. USA 98, 3243–3248.

Shafman, T., Khanna, K.K., Kedar, P., Spring, K., Kozlov, S., Yen, T., Hobson, K., Gatei, M., Zhang, N., Watters, D., et al. (1997). Interaction between ATM protein and c-Abl in response to DNA damage. Nature 387, 520–523.

Shanbhag, N.M., Rafalska-Metcalf, I.U., Balane-Bolivar, C., Janicki, S.M., and Greenberg, R.A. (2010). ATM-dependent chromatin changes silence transcription in cis to DNA double-strand breaks. Cell 141, 970–981.

Shibata, A., Conrad, S., Birraux, J., Geuting, V., Barton, O., Ismail, A., Kakarougkas, A., Meek, K., Taucher-Scholz, G., Löbrich, M., and Jeggo, P.A. (2011). Factors determining DNA double-strand break repair pathway choice in G2 phase. EMBO J. 30, 1079–1092.

Shieh, S.Y., Ikeda, M., Taya, Y., and Prives, C. (1997). DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. Cell 91, 325–334.

Shiloh, Y., and Ziv, Y. (2013). The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. Nat. Rev. Mol. Cell Biol. 14, 197–210.

Sibanda, B.L., Chirgadze, D.Y., Ascher, D.B., and Blundell, T.L. (2017). DNA-PKcs structure suggests an allosteric mechanism modulating DNA double-strand break repair. Science 355, 520–524.

Siliciano, J.D., Canman, C.E., Taya, Y., Sakaguchi, K., Appella, E., and Kastan, M.B. (1997). DNA damage induces phosphorylation of the amino terminus of p53. Genes Dev. 11, 3471–3481.

Singleton, B.K., Torres-Arzayus, M.I., Rottinghaus, S.T., Taccioli, G.E., and Jeggo, P.A. (1999). The C terminus of Ku80 activates the DNA-dependent protein kinase catalytic subunit. Mol. Cell. Biol. 19, 3267–3277.

Skourti-Stathaki, K., Proudfoot, N.J., and Gromak, N. (2011). Human senataxin resolves RNA/DNA hybrids formed at transcriptional pause sites to promote Xrn2-dependent termination. Mol. Cell 42, 794–805.

Smider, V., Rathmell, W.K., Lieber, M.R., and Chu, G. (1994). Restoration of X-ray resistance and V(D)J recombination in mutant cells by Ku cDNA. Science 266, 288–291.

Spagnolo, L., Rivera-Calzada, A., Pearl, L.H., and Llorca, O. (2006). Three-dimensional structure of the human DNA-PKcs/Ku70/Ku80 complex assembled on DNA and its implications for DNA DSB repair. Mol. Cell 22, 511–519.

Spycher, C., Miller, E.S., Townsend, K., Pavic, L., Morrice, N.A., Janscak, P., Stewart, G.S., and Stucki, M. (2008). Constitutive phosphorylation of MDC1 physically links the MRE11-RAD50-NBS1 complex to damaged chromatin. J. Cell Biol. 181, 227–240.

Stiff, T., Walker, S.A., Cerosaletti, K., Goodarzi, A.A., Petermann, E., Concannon, P., O'Driscoll, M., and Jeggo, P.A. (2006). ATR-dependent phosphorylation and activation of ATM in response to UV treatment or replication fork stalling. EMBO J. 25, 5775–5782.

Stracker, T.H., Carson, C.T., and Weitzman, M.D. (2002). Adenovirus oncoproteins inactivate the Mre11-Rad50-NBS1 DNA repair complex. Nature 418, 348–352.

Stucki, M., Clapperton, J.A., Mohammad, D., Yaffe, M.B., Smerdon, S.J., and Jackson, S.P. (2005). MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. Cell 123, 1213–1226.

Sun, Y., Jiang, X., Chen, S., Fernandes, N., and Price, B.D. (2005). A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. Proc. Natl. Acad. Sci. USA 102, 13182–13187.

Sun, Y., Xu, Y., Roy, K., and Price, B.D. (2007). DNA damage-induced acetylation of lysine 3016 of ATM activates ATM kinase activity. Mol. Cell. Biol. 27, 8502–8509.

Sun, Y., Jiang, X., Xu, Y., Ayrapetov, M.K., Moreau, L.A., Whetstine, J.R., and Price, B.D. (2009). Histone H3 methylation links DNA damage detection to activation of the tumour suppressor Tip60. Nat. Cell Biol. 11, 1376–1382.

Swift, M., Reitnauer, P.J., Morrell, D., and Chase, C.L. (1987). Breast and other cancers in families with ataxia-telangiectasia. N. Engl. J. Med. 316, 1289–1294.

Taccioli, G.E., Gottlieb, T.M., Blunt, T., Priestley, A., Demengeot, J., Mizuta, R., Lehmann, A.R., Alt, F.W., Jackson, S.P., and Jeggo, P.A. (1994). Ku80: product of the XRCC5 gene and its role in DNA repair and V(D)J recombination. Science 265, 1442–1445.

Takai, H., Naka, K., Okada, Y., Watanabe, M., Harada, N., Saito, S., Anderson, C.W., Appella, E., Nakanishi, M., Suzuki, H., et al. (2002). Chk2-deficient mice exhibit radioresistance and defective p53-mediated transcription. EMBO J. 21, 5195–5205.

Taylor, A.M., Harnden, D.G., Arlett, C.F., Harcourt, S.A., Lehmann, A.R., Stevens, S., and Bridges, B.A. (1975). Ataxia telangiectasia: a human mutation with abnormal radiation sensitivity. Nature 258, 427–429.

Taylor, A.M., Metcalfe, J.A., Oxford, J.M., and Harnden, D.G. (1976). Is chromatid-type damage in ataxia telangiectasia after irradiation at G0 a consequence of defective repair? Nature 260, 441–443.

Taylor, A.M.R., Lam, Z., Last, J.I., and Byrd, P.J. (2015). Ataxia telangiectasia: more variation at clinical and cellular levels. Clin. Genet. 87, 199–208.

Thorslund, T., Ripplinger, A., Hoffmann, S., Wild, T., Uckelmann, M., Villumsen, B., Narita, T., Sixma, T.K., Choudhary, C., Bekker-Jensen, S., and Mailand, N. (2015). Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. Nature 527, 389–393.

Tibbetts, R.S., Brumbaugh, K.M., Williams, J.M., Sarkaria, J.N., Cliby, W.A., Shieh, S.Y., Taya, Y., Prives, C., and Abraham, R.T. (1999). A role for ATR in the DNA damage-induced phosphorylation of p53. Genes Dev. 13, 152–157.

Toledo, L.I., Altmeyer, M., Rask, M.-B., Lukas, C., Larsen, D.H., Povlsen, L.K., Bekker-Jensen, S., Mailand, N., Bartek, J., and Lukas, J. (2013). ATR prohibits replication catastrophe by preventing global exhaustion of RPA. Cell 155, 1088–1103.

Tresini, M., Warmerdam, D.O., Kolovos, P., Snijder, L., Vrouwe, M.G., Demmers, J.A.A., van IJcken, W.F.J., Grosveld, F.G., Medema, R.H., Hoeijmakers, J.H.J., et al. (2015). The core spliceosome as target and effector of non-canonical ATM signalling. Nature 523, 53–58.

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

van Schendel, R., Roerink, S.F., Portegijs, V., van den Heuvel, S., and Tijsterman, M. (2015). Polymerase Θ is a key driver of genome evolution and of CRISPR/Cas9-mediated mutagenesis. Nat. Commun. 6, 7394.

Walker, A.I., Hunt, T., Jackson, R.J., and Anderson, C.W. (1985). Double-stranded DNA induces the phosphorylation of several proteins including the 90 000 mol. wt. heat-shock protein in animal cell extracts. EMBO J. 4, 139–145.

Walker, J.R., Corpina, R.A., and Goldberg, J. (2001). Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair. Nature 412, 607–614.

Wang, H., Shi, L.Z., Wong, C.C.L., Han, X., Hwang, P.Y.-H., Truong, L.N., Zhu, Q., Shao, Z., Chen, D.J., Berns, M.W., et al. (2013). The interaction of CtIP and Nbs1 connects CDK and ATM to regulate HR-mediated double-strand break repair. PLoS Genet. 9, e1003277.

Weinert, T.A., Kiser, G.L., and Hartwell, L.H. (1994). Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. Genes Dev. 8, 652–665.

Weitzman, M.D., and Weitzman, J.B. (2014). What's the damage? The impact of pathogens on pathways that maintain host genome integrity. Cell Host Microbe 15, 283–294.

Woodbine, L., Neal, J.A., Sasi, N.-K., Shimada, M., Deem, K., Coleman, H., Dobyns, W.B., Ogi, T., Meek, K., Davies, E.G., and Jeggo, P.A. (2013). PRKDC mutations in a SCID patient with profound neurological abnormalities. J. Clin. Invest. 123, 2969–2980.

Yajima, H., Lee, K.-J., and Chen, B.P.C. (2006). ATR-dependent phosphorylation of DNA-dependent protein kinase catalytic subunit in response to UV-induced replication stress. Mol. Cell. Biol. 26, 7520–7528.

Yan, S., and Michael, W.M. (2009). TopBP1 and DNA polymerase-alpha directly recruit the 9-1-1 complex to stalled DNA replication forks. J. Cell Biol. 184, 793–804.

Yaneva, M., Wen, J., Ayala, A., and Cook, R. (1989). cDNA-derived amino acid sequence of the 86-kDa subunit of the Ku antigen. J. Biol. Chem. 264, 13407–13411.

Zampetti-Bosseler, F., and Scott, D. (1981). Cell death, chromosome damage and mitotic delay in normal human, ataxia telangiectasia and retinoblastoma fibroblasts after x-irradiation. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 39, 547–558.

Zha, S., Guo, C., Boboila, C., Oksenych, V., Cheng, H.-L., Zhang, Y., Wesemann, D.R., Yuen, G., Patel, H., Goff, P.H., et al. (2011). ATM damage response and XLF repair factor are functionally redundant in joining DNA breaks. Nature 469, 250–254.

Zhao, H., and Piwnica-Worms, H. (2001). ATR-mediated checkpoint pathways regulate phosphorylation and activation of human Chk1. Mol. Cell. Biol. 21, 4129–4139.

Zhou, Z.-W., Liu, C., Li, T.-L., Bruhn, C., Krueger, A., Min, W., Wang, Z.-Q., and Carr, A.M. (2013). An essential function for the ATR-activation-domain (AAD) of TopBP1 in mouse development and cellular senescence. PLoS Genet. 9, e1003702.

Zhou, Y., Lee, J.-H., Jiang, W., Crowe, J.L., Zha, S., and Paull, T.T. (2017). Regulation of the DNA damage response by DNA-PKcs inhibitory phosphorylation of ATM. Mol. Cell 65, 91–104.

Zou, L., and Elledge, S.J. (2003). Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science 300, 1542–1548.

Zou, L., Cortez, D., and Elledge, S.J. (2002). Regulation of ATR substrate selection by Rad17-dependent loading of Rad9 complexes onto chromatin. Genes Dev. 16, 198–208.

Zou, L., Liu, D., and Elledge, S.J. (2003). Replication protein A-mediated recruitment and activation of Rad17 complexes. Proc. Natl. Acad. Sci. USA 100, 13827–13832.