

# DNA Damage Sensing and Signaling

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## ABSTRACT

Mechanisms have evolved to help protect our genetic material from endogenous and exogenous damage that are vital for an organism's survival. This ability to recognize damaged DNA and simultaneously regulate cell cycle progression and DNA repair is critical for genomic stability, and defects in these pathways are hallmarks of cancer. The DNA damage response pathway is a multi-component signal transduction network that consists of a multitude of proteins whose functions are still under investigation. This review will focus on the current findings in the field including how the DNA damage response influences cancer predisposition and formation.

## 1. INTRODUCTION

Safeguarding an organism's genome from endogenous (reactive oxygen species, abnormal replication intermediates) and exogenous (UV and ionizing radiation, reactive chemicals) sources of DNA damage insures timely and accurate passage of genetic information onto daughter cells. Cells have evolved an elaborate surveillance system to respond to these harmful stimuli. These surveillance pathways are called cell cycle checkpoints, which are activated upon detection of DNA damage, thus allowing the repair of the genetic

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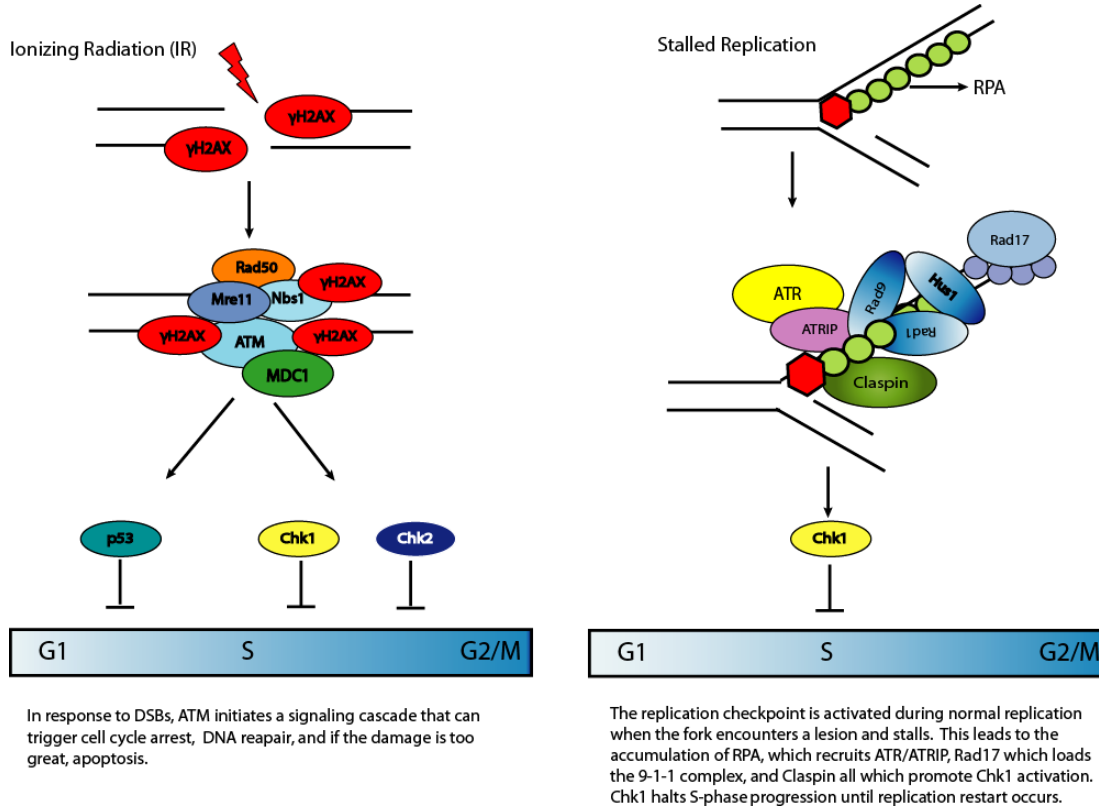
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lesions. Cell cycle checkpoints are critical for detecting damaged DNA and coupling progression through the cell cycle with DNA repair, and if the damage is too great, they can also trigger cell death. Increasing evidence has shown that defects within these checkpoints can lead to genetic instability, a hallmark of cancer cells.<sup>1</sup> Indeed, proteins involved in the DNA damage response and DNA repair, when mutated, cause genetic diseases which predispose individuals to cancer. BRCA1 mutations account for over 50% of familial breast cancer cases, and mutations in ataxia-telangeictasia-mutated (ATM) cause ataxia-telangeictasia (A-T) syndrome, resulting in sensitivity to DNA damaging agents as well as cancer. Recent studies have also provided evidence that the DNA damage response is an anti-cancer barrier, which must be overcome in early neoplastic lesions for tumorigenesis to occur.<sup>2,3</sup> Accurate and timely sensing and repair of DNA lesions is necessary for the cell to survive, and underscores the importance of sensing and signaling DNA damage. This review will focus on the mammalian DNA damage response pathway highlighting recent advances in the field.

## 2. THE DNA DAMAGE CHECKPOINT

The DNA damage response is similar to other signal transduction pathways in that it has several different components that act in concert to activate the checkpoint. It has been suggested that these components consist of sensors, signal transducers, and effectors and recently, to that list has been added mediators.<sup>4</sup> In the course of the cell cycle there are several checkpoints that can be activated in response to DNA damage; the G1/S checkpoint, the intra-S phase checkpoint, and the G2/M checkpoint. Canonically, the DNA damage response is a signal transduction cascade triggered by a series of phosphorylation-dependent events, which activate proteins involved in transducing the DNA damage signal (IR or stalled replication) to different effector proteins which evoke specific effects, namely halting cell cycle progression, activating DNA repair mechanisms and transcription, and triggering apoptosis (Fig. 1). Mediators are placed between the sensors and



**Fig. 1** Activation of the DNA damage response pathway in response to ionizing radiation and replication stress.

signal transducers as their function is to help bring these proteins together to activate the response. However, the DNA damage response is not a linear pathway; DNA damage proteins can also act both upstream and downstream of their respective functions and maintain crosstalk with other proteins. Instead of a linear pathway as previously thought, there is an intricate network of proteins that function independently and dependently to coordinate the response to DNA damage and maintain genomic stability. We will begin to understand this process by first dissecting the definition of sensor proteins in this pathway.

### 3. RECOGNITION OF DNA DAMAGE

It is important that DNA molecules, once damaged, must be recognized to initiate subsequent checkpoint responses. An “official” checkpoint-specific damage sensor is still unknown, though several candidate proteins have been implicated. Genetic systems such as yeast have provided elegant tools to dissect the pathway (Table 1); however, debate still rages as to what the original DNA damage sensor in mammals is. Nevertheless, we will discuss these proteins in their context as sensors: ATM/ATR, the MRN complex, Rad17 and the Rad9-Hus1-Rad1 complex.

Ataxia-telangeictasia mutated (ATM) was first cloned in 1995 as the causative gene for ataxia-telangeictasia (A-T) disorder, which is characterized by cerebellar degeneration, immune system defects and cancer predisposition.<sup>5,6</sup> From this phenotype, ATM has been deduced to be important for the cellular response to double-strand breaks (DSBs); however, the mouse KO is still viable.<sup>7</sup> ATM is a member of the phosphatidylinositol-3-kinase-like family of serine/threonine protein kinases (PIKKs). ATM phosphorylates its substrates on so called SQ/TQ motifs, which is on serine/threonines followed by a glutamine. Like other PIKKs, ATM contains a FAT domain, a phosphoinositide-3,4-kinase (PI3K) domain and a FAT carboxy-terminal (FAT-C) domain. ATM is a nuclear protein that is activated in response to DSBs and possibly chromatin alterations, which has been elegantly shown to dissociate from an inactive

**Table 1. From Yeast to Man: Conservation of the DNA Damage Pathway**

Function	Mammals	<i>S. cerevisiae</i>	<i>S. pombe</i>
<i>Sensors</i>			
PI3K-Kinases	ATM	Tel1	Tel1
	ATR	Mec1	Rad3
Replication Factor C-like	Rad17	Rad24	Rad17
	Rfc2-5	Rfc2-5	Rfc3
PCNA-like	Rad9	Ddc1	Rad9
	Hus1	Mec3	Hus1
	Rad1	Rad17	Rad1
<i>Mediators</i>			
BRCT containing	BRCA1	Rad9	Crb2/Rph9
	MDC1	?	?
	53BP1	?	?
	TopBP1	Dpb11	Cut5
	Claspin	Mrc1	Mrc1
<i>Transducers/Effectors</i>			
Ser/Thr Kinase	Chk1	Chk1	Chk1
	Chk2	Rad53	Cds1
Phosphatase	Cdc25A, Cdc25C	Cdc25	Cdc25
Transcription Factor	p53	?	?

dimer into active monomers through auto-phosphorylation on serine 1981.<sup>8</sup> These active ATM monomers then relocalize to sites of DSBs forming nuclear foci, a common hallmark of most DNA damage responsive proteins.<sup>9</sup> Once at the site of the DSB, ATM phosphorylates several substrates, including H2AX, p53, Chk2, NBS1 and BRCA1. Phosphorylated H2AX ( $\gamma$ H2AX) foci form rapidly at the site and within megabase regions surrounding the DSB, and are thought to be an essential and powerful mechanism for amplifying the damage signal through recruitment of transducer and repair proteins.<sup>10</sup> ATM has been shown to bind to damaged chromatin,<sup>11</sup> further enhancing its role as the sensor protein. However, work recently from Stephen Jackson and colleagues showed that ATM recruitment to DSBs is mediated by NBS1 through a conserved C-terminal motif that binds to ATM.<sup>12</sup> This C-terminal motif is also

found in Ku80 and ATRIP, and was demonstrated to be also responsible for the recruitment of DNA-PK and ATR, respectively. ATM is still the apical kinase that is responsible for the initiation of the DNA damage response to DSBs; however, its function as solely a sensor remains unclear.

Ataxia-telangiectasia-mutated and Rad3 Related (ATR) was first cloned as the human homolog to fission yeast *rad3* and was found to be a member of the PIKK family. ATR shares similar structural similarity with ATM, as well as its function as a serine/threonine kinase; however, ATR is an essential gene as null mutations are embryonic lethal and cells derived from these mice are not viable.<sup>13</sup> Recently a point mutation in the ATR gene was discovered as the cause of an autosomal recessive disorder known as Seckel syndrome, which shares a similar phenotype with Nijmegen breakage syndrome.<sup>14</sup> While ATM is activated in response to DSBs, ATR is activated upon replication stress. During the S-phase, ATR plays a pivotal role in insuring faithful replication, and becomes rapidly activated and binds to chromatin in response to UV, hyperoxia and replication inhibitors.<sup>15–17</sup> How ATR became activated was recently addressed<sup>18</sup> through the hypothesis that RPA coated single-stranded DNA (ssDNA) is a signaling intermediate that is bound by ATR interacting protein (ATRIP), which in turn recruits ATR. Recent data suggest this theory is correct as a C-terminal motif in ATRIP is responsible for the recruitment of ATR in response to replication stress.<sup>12</sup> Therefore, RPA:ssDNA has been postulated to be responsible for activating ATR and is the “signal” required to trigger ATR-dependent checkpoint events.

Next is the highly conserved MRN complex which contains three core proteins: Mre11, Rad50 and Nbs1 that function together to bind to DSBs, recruit ATM, and initiate DNA repair.<sup>19</sup> The MRN complex possesses both 3'-5'-exonuclease and single strand endonuclease activities, DNA binding ability, as well as limited DNA unwinding activity.<sup>20</sup> Electron microscopy and scanning force microscopy have shown that the MRN complex appears as a bipolar structure with a globular head and two long tails. The head consists of two RAD50 ATPase domains (one at the N-terminus, the other at the

C-terminus of Rad50) which are brought together by a flexible hinge region at the center of the protein which contains a CXXC “zinc-hook” that can bind and dimerize with the “zinc-hook” of another Rad50 molecule.<sup>21</sup> Mre11 binds to Rad50 and possesses DNA binding activity which, with the tethering ability of Rad50, can form a flexible tether that can bridge DNA molecules. Nbs1 is the last partner of this important triad, and is thought to stabilize the complex through its interactions with  $\gamma$ H2AX at the site of the DSB, recruit ATM, and participate in ATM-dependent signaling events. Due to its endonuclease and exonuclease activity, the MRN complex has also been postulated to be involved in the early stages of DSB repair by binding to DNA ends and processing them for other repair factors. The MRN complex proteins are essential, as mouse knockouts for each protein is lethal, and in humans hypomorphic mutations in these three proteins cause cancer susceptibility syndromes; Ataxia-Telangeictasia-like disorder (Mre11 and Rad50), and Nijmegen breakage syndrome (Nbs1).<sup>22,23</sup> Patients with these disorders share many similarities with AT patients, presenting with mental retardation, microcephaly, immunodeficiency and predisposition to lymphoid malignancies. Cells derived from these patients display radiation sensitivity, radiation resistant DNA synthesis and chromosome fragility. Both of these disorders recapitulate the AT phenotype and further couple the MRN complex to the function of ATM. Indeed, the MRN complex has been shown to be required for ATM-dependent signaling events, and Nbs1 itself is phosphorylated by ATM on Ser343, which is required for the activation of the S-phase checkpoint.<sup>24</sup> Recently Nbs1 has been shown to have a critical role in ATM recruitment; a C-terminal motif of Nbs1 is required for ATM recruitment to DSBs and has also been shown to promote phosphorylation of ATM substrates such as Chk2, SMC1 and FANCD2, which can further facilitate checkpoint activation and repair.<sup>12,25–28</sup> Thus, instead of acting as a substrate and downstream effector of ATM, the MRN complex is now believed to also function upstream of ATM and may serve as a sensor for DSBs.

Finally, there is the Rad17-RFC and the Rad9-Hus1-Rad1 (9-1-1) complex. In yeast, early genetic screens discovered several mutants

that were sensitive to DNA damage, and were called radiation sensitive or Rad genes. Of these, Rad17, Rad9, Hus1 and Rad1 have been characterized in mammalian and yeast systems and form clamp and clamp-loader complexes similar to that of proliferating cell nuclear antigen (PCNA) and the replication factor complex (RFC). PCNA is a sliding clamp that is loaded onto DNA at sites of ongoing replication and acts as a scaffold to tether other replication proteins. RFC is the clamp-loader responsible for loading PCNA onto DNA. Specifically, Rad17 has been purified and shows similarity with the RFC, with Rad17 replacing the large subunit, forming a clamp loader complex. Human Rad9, Hus1 and Rad1 form a doughnut-shaped trimeric clamp that has been shown to associate with Rad17. More importantly, these proteins have been shown to bind to chromatin after genotoxic stress, and the loading of this so-called 9-1-1 complex onto damaged DNA is Rad17 dependent, paralleling the function of RFC/PCNA in regular replication.<sup>29</sup> This has caused several ideas as to Rad17 and the 9-1-1 complex as a potential DNA damage sensor, as Rad17 loading of the 9-1-1 complex is activated in response to DNA damage. However, recent studies suggest that ATR and the activation of ATR-dependent signaling pathways can occur independently of Rad17 and the 9-1-1 complex, suggesting that these Rad proteins may not be the sole sensors of replication stress.

#### 4. ACTIVATION OF THE DNA DAMAGE RESPONSE

Once DNA damage is sensed and the apical kinases ATM and ATR are activated, they phosphorylate several substrates that initiate a signaling cascade to activate the DNA damage response. These substrates are the so-called mediator and/or transducer proteins: BRCA1, MDC1 and 53BP1 and the kinases Chk1 and Chk2. BRCA1, MDC1 and 53BP1 are mediators in the checkpoint pathway that perform myriad functions to both activate and maintain the damage checkpoint through protein-protein interactions that recruit ATM/ATR substrates to the site of the damaged DNA. Chk1 and Chk2 are phosphorylated and activated by ATM/ATR and transduce the original damage signal and further amplify the

DNA damage response, ensuring activation of surveillance pathways responsible for taking care of the genetic damage.

The breast cancer susceptibility protein 1 (BRCA1) was originally cloned 15 years ago to the long arm of chromosome 17.<sup>30</sup> BRCA1 was found to be a tumor suppressor, as women carrying a mutation in BRCA1 need to lose the wild-type allele for cancer formation. BRCA1 mutations are most commonly found in familial breast and ovarian cancer, with rare mutations found in sporadic breast and ovarian cancer. BRCA1 has two well conserved domains: an N-terminal RING domain and at its C-terminus tandem repeats termed the BRCT domain. Cell lines derived from BRCA1 tumors display sensitivity to DNA damaging agents, defective cell cycle checkpoints and HR repair.<sup>31</sup> BRCA1 knockout mice are embryonic lethal, and ES cells derived from these mice display gross chromosomal rearrangements and defective DNA repair.<sup>32,33</sup> BRCA1, through its RING domain, specifically interacts with BARD1 via its RING domain and forms a heterodimer that has E3 ubiquitin ligase activity. BRCA1/BARD1 ligase activity has been demonstrated *in vitro* and *in vivo*, and catalyzes primarily monoubiquitination and polyubiquitination at K6.<sup>34,35</sup> However, the importance of ubiquitination by BRCA1/BARD1 and its substrates still remains elusive. The BRCT domain has recently been demonstrated to be a phosphor-peptide binding motif by our group and others.<sup>36,37</sup> The BRCT domain interacts with BACH1, a member of the DEAH helicase family, specifically through a phosphor-serine residue (Ser990) that when mutated, abolishes the BRCA1-BACH1 interaction, and disrupts the G2/M checkpoint.<sup>36</sup> Mutations in breast cancer patients in the BRCT domain have been discovered that disrupt this interaction, pointing to the importance of BRCA1 BRCT domain interacting proteins in maintenance of genomic stability. It is believed that through its BRCT domain, BRCA1 can interact with multiple binding partners and thus participates in DNA damage response. While the RING domain of BRCA1 is also important for BRCA1 function, the molecular mechanisms still remain to be resolved.

Our lab and others have discovered MDC1 as an adaptor protein in the DNA damage response pathway.<sup>38–40</sup> MDC1 contains an

N-terminal forkhead-associated (FHA) domain, a phosphothreonine/serine binding motif that is often found in proteins involved in cell cycle, DNA repair and mRNA processing.<sup>41</sup> MDC1 contains a region of 14 conserved repeats (aa1124-1697) that specifically interacts with DNA-dependent protein kinase (DNA-PK), and regulates its autophosphorylation in response to DNA damage, and subsequent DNA repair. Residing in the C-terminus of MDC1 is the tandem BRCT domain, which interacts with  $\gamma$ H2AX.<sup>38</sup> MDC1 interacts with all three members of the MRN complex, SMC1, Chk2, BRCA1 and ATM. Previous studies using MDC1 small interfering RNA (siRNA) in cell lines have bolstered the mediator function of MDC1 as defects in the intra-S phase checkpoint, ATM recruitment, and DNA damaged induced apoptosis is impaired in MDC1 depleted cells.<sup>38-40</sup> Recently, our lab, using a MDC1 deficient mouse model, has shown that MDC1 is a bridging molecule between H2AX and ATM. These three proteins form a positive feedback loop following DNA damage. MDC1 binds to  $\gamma$ H2AX through its BRCT domain and interacts with activated ATM through its FHA domain. Because of these two protein-protein interactions, MDC1 brings ATM in the proximity of H2AX for further H2AX phosphorylation and subsequent accumulation of active ATM at the sites of DSBs (Lou, in press 2006). This loop acts to amplify ATM-dependent signaling events upon DNA damage and facilitates the ATM-dependent phosphorylation events since many ATM substrates are also shown to accumulate at the sites of DSBs in a MDC1/H2AX-dependent manner.

P53 Binding Protein 1 (53BP1) was originally identified in a yeast two hybrid screen for proteins that bound to the DNA-binding region of p53. Similar to MDC1 and BRCA1, 53BP1 contains C-terminal BRCT domains, and a Tudor domain in the middle of the protein. 53BP1 was thought to be the homolog of yeast DNA damage checkpoint Rad9, due to sequence homology. Upon DNA damage, 53BP1 is phosphorylated by both ATM/ATR and relocalizes to the damaged site forming foci.<sup>42</sup> The retention and accumulation of 53BP1 at DSBs requires  $\gamma$ H2AX, and possibly HDAC4.<sup>43,44</sup> Studies utilizing 53BP1-deficient cell lines and 53BP1 siRNA described a defect in the intra S-phase and G2/M checkpoint at low dose of

ionizing radiation, pointing to a role of 53BP1 in cell cycle checkpoint control.<sup>45</sup> 53BP1 deficient mice are viable, but show increased genomic instability and tumor incidence, which suggests that 53BP1 is a bona fide tumor suppressor. This notion is further supported by recent findings from our lab indicating that 53BP1 is a haploinsufficient tumor suppressor as loss of one 53BP1 allele compromised genomic stability and DNA repair.<sup>43,46</sup>

Checkpoint Kinase 1 (Chk1) is a critical messenger of checkpoint control and is activated in response to diverse genotoxic insults. Together with another checkpoint kinase Chk2, it regulates fundamental cellular functions such as DNA replication, cell cycle progression, chromatin restructuring, and apoptosis.<sup>1</sup> Chk1 was first discovered in fission yeast through its ability to complement a cold sensitive *cdc2* mutant. Disruption of fission yeast Chk1 rendered the cells sensitive to UV, bypassing the normal G2/M delay and the cells entering mitosis despite damaged DNA.<sup>47</sup> Unlike yeast, murine Chk1 is essential for development as Chk1 deficient embryos died between embryonic days, 3.5 and 7.5, and Chk1<sup>-/-</sup> ES cells underwent apoptosis.<sup>48</sup> Chk1 is activated in response to DNA damage or replication stress by the ATR and ATM kinases. Chk1 has been demonstrated to be activated upon phosphorylation of two conserved serine residues, Ser317 and Ser 345.<sup>49</sup> Chk1 also requires other factors for efficient activation; these adaptor proteins presumably function to recruit Chk1 to ATR or ATM.<sup>50</sup> Chk1 monitors S-phase progression and G2/M transition by regulating the Cdc25 phosphatase. Cdc25 is crucial for removing the inhibitory phosphorylation on Thr14 and Tyr15 on the cyclin-dependent kinase (cdk), allowing it to become active.<sup>51</sup> In yeast both Chk1 and Cds1 phosphorylate Cdc25 in the presence of DNA damage or replication block, resulting in the inhibition of Cdc25 activity and cell cycle arrest. Human Chk1 has similar functions, i.e. functions mainly mediated through regulation of the human homologues of Cdc25. Chk1-dependent phosphorylation of Cdc25A controls Cdc25A protein levels tightly during normal interphase and also following DNA damage or replication block,<sup>1</sup> and thus regulates cell cycle progression.

Another similar Ser/Thr kinase that also transduces the damage signal to its substrates is Checkpoint Kinase 2 (Chk2). Chk2 is primarily activated by ATM and is important for checkpoint control and apoptosis. Chk2 is the mammalian homolog of budding yeast Rad53 and fission yeast Cds1. In response to DNA damage, Chk2 is phosphorylated and activated by ATM. ATM phosphorylates Chk2 on Thr68, allowing Chk2 to dissociate from inactive dimers to active monomers.<sup>52</sup> This site is not only critical for Chk2 activation but is a docking site for MDC1's FHA domain, as well as Chk2's own FHA domain.<sup>40</sup> Chk2 also autophosphorylates two residues in its activation loop, which is required for full activity, and in its C-terminus S516, which is critical for the induction of apoptosis.<sup>53</sup> As a terminal kinase in the DNA damage signaling pathway, Chk2 phosphorylates several substrates, including BRCA1, Plk1, E2F1, p53 and Cdc25A, affecting the cell cycle, apoptosis and DNA repair. Consistent with Chk2's involvement in several important cellular processes, Chk2 has been postulated as a tumor suppressor. Mutations in Chk2 have been found in a subset of Li-Fraumeni syndrome patients, a syndrome with early childhood onset of tumors that has been mostly linked to p53 mutations.<sup>54</sup> In hereditary breast cancer, a mutation causing the truncation of Chk2 (1100delC) has been found.<sup>55</sup> Despite this evidence, Chk2 null mice are viable, healthy, and are not cancer-prone.<sup>56,57</sup> Of note, these Chk2 deficient mice were extremely resistant to radiation-induced apoptosis, and have given rise to the hypothesis that Chk2 inhibitors may serve as chemopreventative agents.

## 5. ACTIVATION OF CELL CYCLE CHECKPOINTS

Cell cycle checkpoints are the defense mechanism that is initiated by the DNA damage response. Checkpoints allow the cell time to repair by putting the brakes on the cell cycle progression. During this interphase there are specific checkpoints, named the G1/S, intra-S phase, replication, and G2/M checkpoint. We will begin with the important step that starts a cell's fate down the path of mitosis, the transition from G1 to S phase.

## 5.1. G1/S Phase Checkpoint

Primarily the G1/S checkpoint is required to ensure the fidelity of the genome before it undergoes replication. The major effector of this checkpoint is p53, which is activated upon DNA damage by the ATM and Chk2 kinases. Normally p53 is targeted for degradation via the ubiquitin/proteasome pathway by the action of HDM2, an E3 ligase that binds, ubiquitinates and promotes p53 destruction.<sup>58</sup> However, in response to DNA damage, p53 is phosphorylated on Ser20 by Chk2, which disrupts the HDM2-p53 interaction and stabilizes p53.<sup>59</sup> P53 also undergoes phosphorylation by ATM and ATR in response to IR and UV, respectively, on Ser15, which increases p53's transcriptional activity. Once active, p53 activates the transcription of several genes, one of which being p21Cip1/WAF1, a cyclin-dependent kinase (CDK) inhibitor.<sup>60</sup> P21 can bind to CDK2/Cyclin A/E and inhibit its activity, which results in cell cycle arrest in the G1/S restriction point.<sup>61</sup> Therefore, a pathway from DNA damage to ATM/Chk2 to p53/p21 ensures G1/S checkpoint control following DNA damage.

## 5.2. S-phase Checkpoint

The signal for DNA damage during the S-phase can be DNA lesions (DSBs) or impaired DNA synthesis, leading to stalled replication forks. As mentioned before, ATM is activated by DSBs, while ATR becomes active upon replication stress. This leads to two appreciable checkpoints during the S-phase, the intra-S phase checkpoint mediated by ATM, and the replication checkpoint mediated by ATR. Despite this, they both have a common effect, in that they both function to block the firing of origins to delay DNA synthesis allowing for repair. The intra-S checkpoint occurs independently of stalled replication forks. In response to IR, ATM is activated and phosphorylates Chk1, which is the main transducer in this pathway. Chk1 phosphorylates Cdc25A, a serine/threonine phosphatase, which is required for the dephosphorylation of the inhibitory phospho-T14Y15 residues of Cdk2. Cdc25A is phosphorylated by Chk1 on four different residues (Ser123, 178, 278, 292), which

ultimately promotes its degradation via recognition by the F-box protein  $\beta$ -TrcP.<sup>62,63</sup> This leads to cell cycle arrest until the damage is repaired. There is also a parallel pathway that depends on SMC1 for the intra-S-phase checkpoint. SMC1 or structural maintenance of chromosomes 1 is a cohesion protein important for holding the sister chromatids together after replication. SMC1 has been proven to be an important target of ATM in response to IR. SMC1 is phosphorylated by ATM on two residues (Ser957 and Ser966), that when mutated, cause defective intra-S-phase checkpoint and radiosensitization in cells and in knockin mice.<sup>27,64</sup> The molecular mechanism of how SMC1 works in the intra-S-phase checkpoint remains to be determined. SMC1 is not the only protein that has an effect on the intra-S-phase checkpoint; several mediator proteins also play a role. Due to their specific protein-protein interaction motifs, it is thought they act as molecular scaffolds or bridges, thereby recruiting or facilitating downstream targets for phosphorylation by apical kinases at the damage sites. NBS1 and BRCA1 are phosphorylated by ATM in response to IR, and these phosphorylations are important for checkpoint activation as well as SMC1 phosphorylation.<sup>24,65,66</sup> 53BP1 has also been shown to function during the intra-S checkpoint through its ability to affect BRCA1 and Chk2 phosphorylation and foci formation, but recent studies have shown that 53BP1-deficient cells have only a slight or moderate defect in the intra-S-phase checkpoint,<sup>43</sup> suggesting the existence of multiple intra-S phase checkpoint pathways. Indeed, another mediator protein MDC1 also functions in the intra-S-phase checkpoint, as it regulates BRCA1 and SMC1 phosphorylation, as well as interacts and regulates the foci formation of NBS1 and BRCA1.<sup>38–40</sup> Most importantly, depletion of MDC1 results in RDS in response to IR.<sup>38–40</sup> Mediator proteins such as the ones just mentioned have become important in our understanding of the regulation and activation of the intra-S-phase checkpoint.

As mentioned above, the replication checkpoint is also activated in the S-phase upon replication stress. While blocking origin firing, this checkpoint also promotes stabilization of stalled replication forks and restarting of replication following DNA repair. In response to replications stress, ATR is activated and together with

its regulatory subunit, ATRIP, bind to RPA coated single-stranded DNA.<sup>18</sup> Independently, the Rad17 clamp loader recruits and loads the 9-1-1 complex onto chromatin. Additionally, Claspin, a recent protein identified in human and *Xenopus*, associates with the stalled replication forks.<sup>67</sup> Claspin, in response to replication stress, participates in the replication checkpoint by playing a role in Chk1 activation.<sup>68</sup> It is known that the binding of ATR/ATRIP and the 9-1-1 complex occurs independently; however, it seems that they work in concert to phosphorylate and activate Chk1.<sup>29</sup> Together, the recruitment of these proteins allows for the stabilization of the replication forks and full activation of Chk1, which in turn, halts origin firing and further replication through targeting Cdc25A for degradation.

### 5.3. G2/M Checkpoint

The G2/M checkpoint prevents cells from initiating mitosis when they are exposed to DNA damage during G2, or if they harbor unrepaired damage from G1 or the S-phase and they progress into G2. The critical target of the G2/M checkpoint is inhibition of the Cyclin B/Cdk1 complexes, which occurs through inhibition of Cdc25C and also degradation of Cdc25A.<sup>69–71</sup> Cdc25C, like Cdc25A, is a phosphatase whose job is to remove the inhibitory phosphorylation on Thr14 and Tyr15 of Cdk1, resulting in its activation. In response to damage, ATM/ATR are activated and phosphorylate both Chk1 and Chk2, although Chk1 has been shown to be predominant in G2 checkpoint activation. Chk1-deficient cells have defective G2/M checkpoint; however, Chk2 may only play a supporting role as Chk2-deficient cells still maintain an intact G2/M checkpoint.<sup>56,71</sup> Chk1 targets Cdc25A for degradation as previously discussed, and phosphorylates Cdc25C on a 14-3-3 binding site, which promotes its export and sequestration to the cytoplasm. ATM and ATR both function during the G2/M checkpoint. Based on knockout mice studies, it appears that ATM and ATR cooperate in activating early G2 arrest, but ATR is required for late G2 arrest.<sup>72</sup> Mediator proteins again assert their abilities for checkpoint activation, since BRCA1, MDC1,

and to a lesser extent 53BP1, are also required for G2/M checkpoint activation.<sup>40,45,73</sup>

## 6. THE DNA DAMAGE RESPONSE AND CANCER

Maintaining genomic stability is ultimately what cells are supposed to do when they encounter genotoxic stress. Proteins involved in the DNA damage and cell cycle checkpoint response have been shown to be the gatekeepers of the genome, and when lost or mutated can cause genomic instability, a hallmark of cancer cells. Several of these proteins involved in DNA damage response are bona fide tumor suppressors, that when mutated cause inherited cancer susceptibility syndromes (Table 2). Recently, two papers have shown in elegant detail the importance of the DNA damage response pathway in the formation of cancer. Bartkova *et al.* and Gorgoulis *et al.* demonstrate that DNA damage pathways are activated during uncontrolled DNA replication in cells with overexpression of oncogenes.<sup>2,3</sup> The activation of normal DNA damage response in these precancerous cells should arrest cell cycle progression and/or activate apoptosis. The authors postulate that this in turn might cause a selection pressure to inactivate proteins involved in the DNA damage response so that full-blown cancer cells can emerge in the absence of these normal cell cycle brakes. Thus, a model may be that in early precancerous lesions the DNA damage response is activated and many of these

**Table 2. Inherited Cancer Susceptibility Syndromes**

Syndrome	Gene	Nature of Mutation	Cancer Phenotype
Ataxia-Telangeictasia (A-T)	ATM	Null and Hypomorphic	Lymphoid tumors, T-cell Leukemias
Ataxia-Telangeictasia-like Disorder (ATLD)	Mre11	Hypomorphic	None Reported
Nijmegen-Breakage Syndrome (NBS)	Nbs1	Hypomorphic	B-Cell Lymphomas, Leukemias
Seckel Syndrome	ATR	Hypomorphic	None Reported
Familial Breast and Ovarian Cancer	BRCA1	Missense, Frameshift	Breast and Ovarian Cancer

lesions will not progress into malignant tumors. However, in some of these precancerous cells, the normal DNA damage response may be inactivated due to random mutations, thus leading to the further accumulation of genetic instability and eventually tumorigenesis. More work investigating these hypotheses begs attention as we can further understand how cancer develops.

## 7. SUMMARY

In conclusion, the DNA damage response is an intricate network of signaling pathways whose sole purpose is to protect the genome. Deregulation of this response can lead to genomic instability and tumorigenesis, as evidenced by inherited cancer susceptibility syndromes associated with these proteins, and their inactivation or deregulation in cancer cells. Current and future research directed at understanding these pathways can lead to the elucidation of tumorigenesis and possible therapeutic applications that can ameliorate the looming threat that cancer is across the world.

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