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Kinases that Control the Cell Cycle in Response to DNA Damage: Chk1, Chk2, and MK2

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Summary

In response to DNA damage eukaryotic cells activate cell cycle checkpoints - complex kinase signaling networks that prevent further progression through the cell cycle. In parallel to implementing a cell cycle arrest, checkpoint signaling also mediates the recruitment of DNA repair pathways. If the extent of damage exceeds repair capacity, additional signaling cascades are activated to ensure elimination of these damaged cells. The DNA damage response has traditionally been divided into two major kinase branches. The ATM/Chk2 module is activated after DNA double strand breaks and the ATR/Chk1 pathway responds primarily to DNA single strand breaks or bulky lesions. Both pathways converge on Cdc25, a positive regulator of cell cycle progression, which is inhibited by Chk1 or Chk2-mediated phosphorylation. Recently a third effector kinase complex consisting of p38MAPK and MK2 has emerged. This pathway is activated downstream of ATM and ATR in response to DNA damage. MK2 has been shown to share substrate homology with both Chk1 and Chk2. Here we will discuss recent advances in our understanding of the eukaryotic DNA damage response with emphasis on the Chk1, Chk2, and the newly emerged effector kinases p38MAPK and MK2.

Introduction

To maintain genomic integrity and faithful transmission of fully replicated and undamaged DNA during cell division, eukaryotic organisms evolved a complex DNA surveillance program. Prior to mitosis cells progress through G₁/S, intra-S and G₂/M cell cycle checkpoints [1–3]. Checkpoint signaling is activated in response to incomplete DNA replication due to stalled replication forks, and damaged DNA induced by both internal and external sources such as UV light, ionizing radiation, reactive oxygen species or DNA damaging chemotherapeutic agents. Active checkpoints prevent further progression through the cell cycle. If the genotoxic insult exceeds repair capacity, additional signaling pathways, leading to cell death, presumably via apoptosis, are activated [1,4,5].

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We apologize to our colleagues for the omission of many seminal contributions to the field, and their references, owing to space constraints.

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The canonical DNA damage response network has traditionally been divided into two major kinase signaling branches utilizing the upstream PI3-Kinase-like kinases ATM and ATR. These kinases control the G₁/S, intra-S and G₂/M checkpoints through activating their downstream effector kinases Chk2 and Chk1, respectively [1–3]. We have recently identified the p38MAPK/MAPKAP-K2 (MK2) complex as a third checkpoint effector module that operates parallel to Chk1 and is activated downstream of ATM and ATR [6,7].

Upstream Kinase Activation

How are DNA lesions, such as double strand breaks (DSBs), initially detected and transduced into signals activating ATM and/or ATR? The earliest events likely involve alterations in chromatin structure [8–10], but the biochemical details underlying this are poorly understood. Among the earliest events is recruitment of a mediator complex consisting of Mre11, Rad50 and Nbs1 (MRN), and phosphorylation of a variant H2A histone – H2AX – in the DNA near the break, extending for distances up to several megabases [11]. Working together, MRN and phosphorylated H2AX (γ H2AX) act as a signal amplifier that recruits additional signaling molecules to the DSB lesion and dramatically enhances local activation of ATM. The MRN complex serves as an initial DSB sensor, at least one component of which (Nbs1) localizes to the break in an H2AX-independent manner [12,13] and facilitates recruitment of ATM to the site of the lesion [14].

Inactive ATM appears to exist as a multimer that dissociates into active monomers upon activation [15]. Bakkenist and Kastan showed that human ATM underwent a rapid phosphorylation on Ser-1981 following as little as 0.5 Gy of IR and that phosphorylation at this site appeared to correlate with dissociation of ATM into active monomers based on cross-linking studies and co-immunoprecipitation experiments. Furthermore, mutation of Ser-1981 to Ala was shown to block human ATM dissociation in 293 cells, and had a dominant inhibitory effect on both the IR-induced G₂/M and S-phase checkpoints when transfected into HeLa cells [15]. However, Pellegrini et al. [16] generated a murine Ser-1987 to Ala knock-in mouse (corresponding to human Ser-1981) and showed that ATM activation and checkpoint responses after IR appeared to be normal. Thus, the precise role of Ser-1981 phosphorylation in ATM activation remains somewhat unclear. Intriguingly, Price and colleagues recently reported that acetylation of Lys-3016 in the FATC domain of human ATM appears to be required for ATM activation following IR, and acetylation at this site shows identical kinetics as Ser-1981 phosphorylation [17,18]. The acetyl transferase likely responsible for ATM acetylation at Ser-3016 is Tip60, a recently described haploinsufficient tumor suppressor that in addition to forming complexes with ATM, also binds to the MRN complex at double strand breaks via its-cofactor Trrap and is required for efficient loading of Brca1 and 53BP1 onto damaged chromatin [19–21]. RNAi-mediated Tip60 depletion or expression of a dominant negative Tip60 mutant was shown to result in diminished ATM activation and reduced cell survival following DNA damage, suggesting that acetylation of ATM, rather than auto- or cross-phosphorylation, may be the critical activating event [18].

Active ATM then phosphorylates the variant histone H2AX on a critical Ser residue, Ser-139, yielding γ -H2AX [3,11]. The phosphorylation site on H2AX, corresponding to the sequence S-Q-E-Y, is subsequently recognized by the phosphopeptide-binding BRCT domains of another mediator protein, MDC1 [22–24], which in turn gets phosphorylated by ATM at multiple sites, allowing MDC1 to recruit an E3 ubiquitin ligase complex through binding to the ring-finger protein RNF8. The E3 ubiquitin ligase RNF8, together with the E2 enzyme Ubc13, in turn mediates ubiquitination of H2AX, and possibly H2A or other chromatin-associated proteins, ultimately recruiting a complex consisting of Rap80, Abraxas and BRCA1 via the ubiquitin interaction motif in Rap80 [25–28]. BRCA1, along with 53BP1, which is also recruited downstream of RNF8, play important roles in DNA repair via homologous

recombination (HR) and non-homologous end joining (NHEJ), respectively [29,30]. In addition, MDC1 also undergoes phosphorylation by CK2 after DNA damage to generate a phospho-motif on MDC1, which binds directly to Nbs1. This CK2-dependent recruitment of Nbs1 seems to be important for stabilizing MRN at the DSB [31,32]. In addition, Nbs1 can also immobilize ATM at the site of the DSB via direct binding of ATM to a C-terminal ATM interaction motif on Nbs1 [14]. Thus, MDC1, through ATM- and CK2-directed phosphorylation, promotes tighter binding of the MRN complex and ATM at the site of the DSB, forming a powerful ATM auto-amplification loop.

When replication forks stall during S-phase, MCM helicases continue to unwind the DNA template, resulting in exposure of stretches of ssDNA, which are rapidly coated by the ssDNA-binding protein RPA [33]. RPA bound to ssDNA recruits ATR through direct binding of its regulatory subunit ATRIP to RPA [34]. The precise biochemical mechanism responsible for activating ATR following its recruitment to ssDNA lesions is largely unknown, although the process appears to involve ATR-ATRIP interaction with an additional DNA-associated protein complex composed of Rad9-Rad1-Hus1 (9-1-1) and TopBP1. The 9-1-1 complex functions as a clamp, encircling the DNA, and recruits the BRCT domain-containing protein TopBP1 in a phospho-dependent manner (for a detailed review see [35]). Dunphy and colleagues have shown in *Xenopus* extracts that TopBP1 is required for proper ATR activation and replication checkpoint control [36]. Although ATR is thought to be activated primarily by single stranded DNA lesions, under certain circumstances it can also be activated downstream of, DSBs. If cells encounter DSBs in late S-phase or G₂, ATR is activated in an ATM-dependent manner likely through the generation of RPA coated ssDNA by the endo- and/or exonuclease activity of Mre11/CtIP complexes [37,38].

Downstream Effector Kinases and their Targets

The locally increased ATM activity is believed to be important for efficient phosphorylation of ATM substrates including the downstream effector kinase Chk2 and the prominent tumor suppressor protein p53. Several groups have reported that within the nucleus ATM phosphorylates Chk2 on Thr-68 in a Ser/Thr cluster following IR *in vitro* and *in vivo* [39–41]. This phosphorylation is thought to be involved in Chk2 activation, since Thr-68-Ala mutation results in impaired Chk2 activation. However, phosphorylation on Thr-68 does not seem to be essential for Chk2 activation, since Thr-68-Ala mutation does not completely abolish IR-induced Chk2 activation, when the mutant is expressed on a wildtype background [41]. In fact, it has been proposed that phosphorylation on Thr-68 triggers a chain of additional autophosphorylation events on Thr-383 and Thr-387 in the activation loop, which ultimately result in Chk2 activation [39,42]. In addition, oligomerization of Chk2 is believed to increase the kinase activity of Chk2 [43]. Piwnicka-Worms and colleagues recently confirmed Thr-68, 383 and 387 as Chk2 phosphorylation sites and also identified Ser-516 as an additional Chk2 phosphorylation site [39]. Using Chk2-proficient cells transfected with a kinase dead Chk2 expression construct, Piwnicka-Worms and colleagues demonstrated that exogenous Chk2 gets phosphorylated on Thr-68, 383 and 387. Ser-516 was not phosphorylated, suggesting autophosphorylation *in cis* at this site. However, when Chk2-deficient cells were transfected, only Thr-68 was found to be phosphorylated, suggesting that Thr-383 and 387 are indeed Chk2 autophosphorylation sites that can be phosphorylated *in trans*. Interestingly, Thr-68 was also found to be phosphorylated in response to IR even in ATM-deficient cells and in cells pretreated with caffeine, suggesting that kinases other than ATM, ATR or DNA-PKcs might be involved. Chk2 expressed in *E. coli* was found to be phosphorylated on Thr-68, 383, 387 and Ser-516, which correlated with increased activity even in the absence of IR. Similar findings were made when Chk2 was overexpressed in 293T cells or Chk2-deficient MEFs. These observations are in agreement with data from Gilbert *et al.*, who suggested that locally increased concentrations of Rad53, the budding yeast homologue of Chk2, results in increased Rad53 kinase activity,

as a result of *trans*-phosphorylation [44]. Moreover, Chk2 oligomerization was found to be impaired when Thr-157 was mutated to Ile, a mutation in the FHA domain of Chk2, found in a subset of Li-Fraumeni syndrome patients [39]. Notably, when either wildtype or Chk2-Thr157Ile were co-expressed in Chk2-deficient MEFs, Chk2-Thr157Ile displayed markedly reduced phosphorylation on Thr-68, 383 and 387 in the absence of IR. Whether this lack of phosphorylation is a reflection of impaired oligomerization or merely the result of gross structural irregularity remains elusive.

The effector kinase Chk1 is one of the best-studied ATR substrates and its effective activation by ATR-mediated phosphorylation requires the presence of the mediator protein claspin [45–47]. ATR phosphorylates Chk1 on two serine residues that lie outside the kinase domain, Ser-317 and 345, resulting in a marked increase in Chk1 activity [48]. It remains unclear whether ATR kinase activity is required to recruit claspin to RPA-coated ssDNA. However, in the context of ssDNA generated during S-phase progression, ATR-mediated phosphorylation of the ssDNA binding protein Rad17 is required for claspin recruitment [49].

Besides activation of Chk1 and Chk2 following DNA damage, proteins involved in many different cellular processes, such as DNA repair and replication, RNA metabolism, nonsense mediated decay, regulation of translation, chromatin remodeling, circadian rhythm, insulin signaling, mitosis, regulation of transcription and MAPK signaling have been identified as putative ATM and ATR substrates by screening approaches over recent years [3,50]. Modulation of most of these events by ATM and ATR remain largely unexplored.

Both, the ATM/Chk2 and the ATR/Chk1 pathway converge to inactivate members of the Cdc25 family of dual-specificity phosphatases, which play an important role in driving dividing cells through the cell cycle [51]. At the G₁/S and G₂/M transition these enzymes remove critical inhibitory phosphorylations on Cdk/Cyclin complexes, the most important of which is dephosphorylation of Tyr-15 in the ATP-binding loop of Cdks 1 and 2. Of the three known Cdc25 family members Cdc25A is believed to be a critical Chk1 substrate for the intra-S phase checkpoint, marking Cdc25A for rapid destruction by the 26S proteasome [51,52]. Recent data suggest a role for Cdc25A at the G₂/M boundary, as well [53]. The role of Chk2 in the regulation of Cdc25A is less clear. One report suggests that Chk2 may perform similar functions under certain conditions [54], although Harper and colleagues have recently brought this into question [55]. Following intra S-phase checkpoint activation, Cdc25A is phosphorylated on the known Chk1 site Ser-76 and subsequently undergoes SCF^{β-TRCP}-dependent ubiquitination and proteasomal degradation [52]. Recognition of Cdc25A by β-TRCP occurs via a noncanonical phosphodegron in Cdc25A that, besides pSer-76, involves pSer-79 and pSer-82, sites that do not match the basophilic Chk1 substrate motif [52]. In addition to the intra S-phase checkpoint, which is governed by Chk1, Cdc25A is also targeted for ubiquitin-mediated degradation during G₁. Piwnicka-Worms and colleagues recently demonstrated that GSK3β phosphorylates Cdc25A on Ser-76 in G₁ after priming of Cdc25A by Plk3-mediated phosphorylation of Thr-80 [56]. Ser-76 phosphorylation by Chk1 or GSK3β appears to be a requirement for subsequent Ser 82 phosphorylation, which in turn is essential for Cdc25A recognition by SCF^{β-TRCP} [2, 52,56–58]. At the G₂/M boundary Cdc25B has been reported to be the starting phosphatase that initially activates Cdk1/CyclinB, which activates Cdc25C, creating an autoactivation loop [51]. Cdc25B-1/2 and Cdc25C are functionally inactivated upon phosphorylation on Ser-309/323 and Ser-216, respectively, leading to 14-3-3 binding and nuclear exclusion [51, 59]. In addition, Chk1 has also been shown to directly target and activate Wee1, the kinase directly responsible for the inhibitory phosphorylation of Cdk1 and 2 on Tyr-15. Dunphy's group, using *Xenopus* extracts, and Piwnicka-Worms' group, using HeLa cells, showed that Chk1-dependent Wee1 phosphorylation on Ser-549 (*Xenopus*) and Ser-642 (human) promotes Wee1 association with 14-3-3, directly increasing its catalytic activity [60,61]. Mutation of

Ser-549/642 to Ala prevented 14-3-3 binding and substantially reduced the ability of Wee1 to phosphorylate Cdk1. Furthermore, replacement of endogenous Wee1 with Wee1 carrying a Ser-549 Ala mutation in *Xenopus* egg extracts, or expression of the Ser-642 Ala mutant in HeLa cells, attenuated a Chk1-induced cell cycle delay [61]. Similar observations were reported by Nurse and colleagues using *S. pombe* as a model system [62].

A Novel cell Cycle Checkpoint Kinase Pathway that Integrates Global Stress Responses with DNA Damage

It has become increasingly clear that the DNA damage response network extends beyond the canonical ATM/Chk2 and ATR/Chk1 signaling modules, to include connections to pathways as diverse as those involving PI 3-kinase/AKT, IKK/NF κ B, and various MAP Kinases (MAPKs) [63–66]. Multiple lines of evidence suggest an increasingly important role for the p38MAPK pathway and its downstream effector kinase MK2.

The p38MAPK/MK2 signaling complex is considered to be a general stress response pathway, which is activated in response to a variety of extrinsic and intrinsic stimuli including osmotic stress, heat shock, various toxins, UV and ionizing radiation, reactive oxygen species (ROS), cytokines, loss of centrosome integrity and DNA damage [67]. p38MAPK activation drives a plethora of changes in transcription, protein synthesis, cell surface receptor expression, and cytoskeletal structure, ultimately affecting cell survival and apoptosis.

There are 4 p38MAPK isoforms denoted α , β , γ , and δ [67]. Splice variants exist for p38 α and β , giving rise to a total of six p38MAPK isoforms [68]. p38 α was first identified as a tyrosine-phosphorylated protein in extracts of LPS-treated macrophages and was subsequently shown to have significant homology with the yeast stress kinase HOG1 [68]. Additional members of the p38MAPK family were cloned by homology. p38 α appears to be ubiquitously expressed, with high levels in leukocytes, liver, spleen, bone marrow, thyroid, and placenta. In contrast, p38 β shows some specificity for brain and heart, while p38 γ is expressed at highest levels in skeletal muscle. p38 δ has been reported to be expressed at highest levels in the lung, kidney, gut, salivary gland and endocrine organs such as the testis, ovary, adrenal-, and pituitary gland [68]. Intriguingly, p38 α exists in a stable complex with its downstream substrate, the kinase MK2 [69–72], and the stability of p38 α has been reported to depend on the presence of MK2. Gaestel and colleagues reported modestly reduced levels of p38MAPK in MK2-deficient mice [73]. Whether similar complexes exist with p38 β , γ , or δ is less clear. As with all known MAPKs, p38MAPKs are activated by dual phosphorylation on a T-X-Y motif in the activation loop through the action of MAP kinase kinases (MAPKKs, or MKKs). MAPKKs in turn are activated by a plethora of different MAP kinase kinase kinases (MAPKKKs, or MEKKs).

Many upstream MAPKKKs are known to participate in activation of the p38MAPK cascade including MEKK1/4, ASK1, and TAO kinases, depending on the particular type of initiating stimulus, which the cell is responding to. Using functionally p53-deficient HeLa cells, Cobb and colleagues showed that the MAPKKK TAO, activated p38 α in an ATM-dependent manner in response to UV, IR and hydroxyurea treatment [74]. Davis and colleagues, on the basis of overexpression studies, mouse knock-outs, and siRNA experiments, have shown that the MAPKKs MKK3 and 6 are the major upstream activators of p38 α , β 2, and γ in response to hyperosmolar stress and signaling by the pro-inflammatory cytokines TNF α and IL-1, while MKK4 has a redundant role along with MKK3 and 6 in p38MAPK activation by UV-irradiation [75,76]. MKK4 is a known JNK MAPKK, suggesting that it may function as a signaling hub to integrate JNK and p38MAPK signaling [77]. The upstream MAPKK activators of p38MAPK in response to genotoxic agents other than UV has not been reported.

Among the known substrates of p38MAPK are a number of transcription factors, including ATF1/2/6, MEF2A/C, p53, SAP1, STAT1, Gadd153 and Max, as well as the MAPK activated protein kinases MSK1 and 2, MNK1 and 2 and MK2, 3, and 5 (for detailed reviews see [67, 78,79]).

Over the last decade a number of observations have been published that point to a critical role for the p38MAPK module as an integral part of the DNA damage response network. Besides UV, p38 α and β have been shown to be activated by other, more DNA damage-specific agents, such as cisplatin, doxorubicin and temozolomide [6,7,74,80,81]. Hirose *et al.* [80] observed a p38MAPK-dependent G₂/M arrest following temozolomide exposure in a mismatch repair-proficient human glioma cell line. p38MAPK signaling was associated with nuclear inactivation of Cdc25C, and RNAi-mediated knock down of p38 α or pharmacologic inhibition of p38 α and β reversed these effects. Mikhailov *et al.* [81] were able to show that p38MAPK signaling is activated upon treatment of PtK₁ cells with topoisomerase II inhibitors, and resulted in a late G₂/early prophase (so-called ‘antephase’) delay prior to mitotic entry. This delay could be overridden upon pharmacological inhibition of p38 α and β . In this cell type, p38 α and β were not required for normal mitotic progression in the absence of topoisomerase inhibition, or for the spindle assembly checkpoint. Although not a direct genotoxin, Sun *et al.*, examined H-ras^{V12} oncogene-induced replicative stress, and found that p38MAPK mediated activation of MK5 is required for H-ras^{V12}-induced senescence in a murine model of DMBA-induced skin carcinogenesis. Induction of p38MAPK-dependent senescence was mediated by MK5-induced phosphorylation of p53 on Ser-37 [82], and suggests that the p38MAPK pathway may function as a tumor suppressor in this context. Further insight into the DSB-mediated activation of p38MAPK came from the study of VDJ recombination in DN3 thymocytes. During T-lymphocyte maturation, cells undergo physiological induction of DSBs by RAG recombinase, followed by DSB repair in a DNA-PKcs-dependent manner. Pedraza-Alva *et al.* [83] used wild-type (WT) DN3 thymocytes, as well as Rag^{-/-} and Scid DN3s. WT cells were found to activate p38MAPK when DSBs were generated during VDJ recombination, and concomitantly underwent a G₂/M arrest. While Rag^{-/-} cells do not accumulate unresolved DSBs, Scid cells do so, due to a lack of DNA-PK activity, which is mutated on the Scid background. Since DNA-PK is a key component of the NHEJ repair machinery, Scid thymocytes are unable to repair the Rag induced DSBs. When the authors compared levels of activated p38MAPK in Rag^{-/-} and Scid thymocytes, they found increased levels of active p38MAPK only in Scid cells. The authors further describe the accumulation of both phospho-p38MAPK and phospho-p53 in Rag^{-/-} thymocytes expressing constitutively active MKK6, and suggested that p38MAPK, acting through p53, was responsible for the G₂/M checkpoint observed in these cells. Phosphorylation of p53 on Ser-18 and Ser-389 (corresponding to human Ser-15 and Ser-392) depended on p38MAPK and was abolished by the addition of the p38MAPK inhibitor SB203580. This study indicated that the p38MAPK pathway is activated in response to the accumulation of DSBs independently of DNA-PK, and may be involved in a p53-dependent G₂/M arrest.

Kurosu and colleagues examined the role of p38MAPK in Burkitt’s lymphoma cells treated with the topoisomerase II inhibitor etoposide. This DNA damage-specific approach revealed that p38MAPK is activated following induction of DNA DSBs after topoisomerase II inhibition. In subsequent experiments using pharmacological inhibition of p38MAPK, as well as inducible expression of a dominant negative p38MAPK mutant, they were able to demonstrate that p38MAPK is required for an etoposide induced G₂/M arrest and that abrogation of this checkpoint resulted in increased apoptosis [84].

Fornace and colleagues made the important observation that p38 α and β signaling is necessary for the initiation of a G₂/M arrest after low-dose UV-irradiation, with p38 α playing a more prominent role. Application of the specific p38MAPK inhibitor SB202190, or reduction of

p38 α and/or β levels using antisense oligonucleotides reversed this effect resulting in sustained mitotic activity for the first several hours after irradiation, although the mitotic index fell to similar levels as those observed in control cells 8 hrs later [85]. This group further reported that p38MAPK-containing immunoprecipitates contained a kinase activity capable of directly phosphorylating Cdc25B and C to generate critical 14-3-3 binding sites, an effect which was interpreted as evidence that p38MAPK could directly generate 14-3-3-binding sites on Cdc25B/C. Ben-Levi *et al.* [69], however, demonstrated that p38 α forms a tight nuclear complex with its downstream substrate MK2 and that upon activation of p38MAPK in this complex, p38MAPK phosphorylates and activates MK2. This phosphorylation is essential for the nuclear export of the p38MAPK/MK2 complex in response to arsenite, and presumably other activating stimuli [69,70,78]. Based on a long-standing interest in p38MAPK and its substrates, we determined the optimal sequence motifs phosphorylated by p38 α and its downstream effector kinase MK2 [6] using oriented peptide library screening. The optimal p38MAPK motif requires the presence of a Pro immediately C-terminal to the phospho-acceptor Ser or Thr residue, and does not conform to the critical 14-3-3- binding sites on Cdc25B or C. In contrast, the optimal phosphorylation motif found for MK2, ([L/I/F]-X-**R**-[Q/M/S/T]-X-[S/T]- ϕ , where [S/T] denotes the phosphoacceptor residue and ϕ indicates a hydrophobic amino acid, perfectly matches the known pSer-323 14-3-3 binding site in Cdc25B, as well as pSer-216 14-3-3 binding site in Cdc25C [6]. Manke *et al.* went on to demonstrate that recombinant MK2 from bacteria directly phosphorylated Cdc25B on Ser-323, thereby generating a 14-3-3 binding site on this molecule, and that RNAi-mediated knockdown of MK2 abolished the increase in 14-3-3 binding to cdc25B and C upon UV irradiation. Ducommun's lab recently mapped the p38MAPK and MK2 phosphorylation sites on Cdc25B by mass spectrometry, providing independent confirmation that MK2 generates the Ser-323 binding site for 14-3-3 [86]. Manke *et al.* found that MK2-depleted cells were defective in both the G₁/S and G₂/M checkpoints after irradiation, rendering them more sensitive to UV-induced cell death as a consequence of mitotic catastrophe following checkpoint dysfunction. Together, these observations indicated that MK2 is the critical checkpoint effector that functions downstream of p38MAPK to arrest cell cycle progression in response to UV irradiation.

This role as a cell cycle regulator for MK2 appears to be highly conserved among eukaryotes. Lopez-Aviles *et al.* [87] showed that overexpression of *Srk1*, the *S. pombe* homologue of human MK2, caused a delay in mitotic entry while cells lacking *Srk1* were shown to enter mitosis prematurely. These cell cycle regulatory effects resulted from *Srk1* interaction with, and phosphorylation of, Cdc25 at sites at least partially identical to those phosphorylated by Chk1 and Cds1, the *S. pombe* homologue of Chk2. Following *Srk1*-dependent phosphorylation, Cdc25 bound to Rad24, one of two *S. pombe* 14-3-3 proteins, leading to Cdc25 nuclear exclusion, stabilization, and catalytic inhibition, essentially recapitulating the identical MK2-Cdc25-14-3-3 pathway seen in mammalian cells. In contrast to mammalian cells, however, *Srk1*-deficient *S. pombe* cells did not display increased sensitivity to UV irradiation, although depletion of Chk1 rendered them highly sensitive. Thus, in fission yeast, it appears that *Srk1* functions during the normal G₂ phase of an unperturbed cell cycle, but is not part of the UV-induced DNA damage cell cycle checkpoint response. *Srk1* activation towards Cdc25 is controlled by *Stk1*, the *S. pombe* homologue of p38MAPK, with which *Srk1* forms a stable complex in the resting state [88].

The budding yeast *S. cerevisiae* contains two potential MK2 homologues, *rck1* and *rck2*. In an unbiased global screen of nearly 5,000 haploid deletion strains, Begley *et al.* observed that loss of *rck2*, resulted in increased UV sensitivity, indicating that this MK2 homologue is physiologically important for the cellular response to UV-induced damage in budding yeast [89].

Mammalian MK2, like the fission yeast homologue, may also control cell cycle progression in response to stimuli other than direct DNA damage. Huard *et al.* [90] recently reported that the viral protein R (VPR) of HIV, which induces a G₂ arrest in lymphocytes to block clonal T-cell expansion, does so by activating MK2. Similar to our observations on UV-activation of MK2, this VPR-activated form of MK2 was found to mediate phosphorylation of Cdc25C on the known Ser-216 14-3-3-binding site. Okamoto *et al.* reported that HIV gp120, which induces a G₁ arrest in neuronal stem cells, and may contribute to HIV-induced dementia, also functions through the activation of the p38MAPK/MK2 pathway. These HIV-related findings suggest that global cellular stresses from viral infection events trigger MK2-mediated cell cycle arrest, likely independently of damage to the DNA backbone or bases [91].

When DNA is intentionally damaged by anti-cancer chemotherapeutic agents in mammalian cells, activation of the p38MAPK pathway appears to require ATM and ATR. Using ATM and ATR-deficient human cells as well as pharmacological inhibition of ATM and ATR, Reinhardt *et al.* recently showed that the p38MAPK/MK2 module operates downstream of ATM and ATR in response to cisplatin, camptothecin and doxorubicin [7]. Interestingly, p38MAPK/MK2 activation in response to UV was shown to be independent of ATM and ATR, suggesting that UV likely causes cellular lesions other than DNA damage that result in p38MAPK activation. Importantly, cisplatin- and doxorubicin-mediated activation of the p38MAPK/MK2 module was independent of Chk1, and conversely, Chk1 activation was independent of MK2 activity, indicating that the ATR/ATM-p38MAPK-MK2 pathway functions in parallel with the ATR-Chk1 pathway. The notion that MK2 operates in a synergistic parallel pathway to the classical checkpoint effectors Chk1 and Chk2 is further supported by the observation that *S. cerevisiae* MK2 homologues *rck1* and *rck2* can function as extragenic suppressors of *S. pombe* cell cycle checkpoint mutations in *chk1*, as well as mutations in *rad1*, *rad9*, *rad17*, and *rad26* [92], reversing the sensitivity of these strains to UV and IR, and to the replication blocker hydroxyurea. The dependence on the canonical DNA damage response kinases ATM and ATR for p38MAPK/MK2 activation following genotoxic stress has now been confirmed independently [74,93].

To further investigate whether there was a context-dependent role for MK2 in cell survival following genotoxic stress, Reinhardt *et al.* recently investigated the effects of MK2 depletion in p53-proficient and p53-deficient MEFs [7]. MK2 activity appeared to be dispensable for cellular survival following cisplatin or doxorubicin in p53-proficient cells. However, MK2 depletion in p53-deficient MEFs resulted in an increased sensitivity to both cisplatin and doxorubicin. This synthetic lethality between the prominent tumor suppressor p53 and MK2 was due to the inability of p53-deficient cells expressing MK2 shRNA to execute functional G₁/S and G₂ cell cycle checkpoints following doxorubicin or cisplatin. Similar results were obtained when allograft tumors derived from p53-deficient, H-ras^{V12}-transformed MEFs were examined for chemosensitivity in a nude mouse model *in vivo*. Knockdown of MK2 in these tumors resulted in dramatically increased sensitivity to systemic chemotherapeutic treatment with cisplatin or doxorubicin. Intriguingly, MK2-depleted tumors grew more rapidly than control tumors, indicating a potential role of MK2 as a negative regulator of the unperturbed cell cycle in tumor cells, and a potential tumor suppressor.

Although several groups have now confirmed members of the Cdc25 family as physiological MK2 substrates following genotoxic stress [6,7,86,90], the question remains whether other checkpoint-relevant substrates for MK2 exist *in vivo*. It is intriguing to speculate that a pool of substrates exists that is *not* shared by Chk1 and MK2. For example, although both kinases recognize and phosphorylate the same optimal amino acid motif sequence, and both are present in the nucleus, active MK2 has been shown to translocate to the cytoplasm, in response to osmotic stress, arsenite exposure, and cytokine stimulation [69,70]. It is therefore intriguing to speculate that MK2 might control a late cytoplasmic component of the DNA damage

response. Among the known targets of MK2 following cytokine stimulation are a number of proteins involved in post-transcriptional regulation of mRNA and protein translation, including Ago2, a protein critical for the RNAi pathway [94–98]. Could MK2-imposed control of mRNA metabolism, microRNA function, and protein translation also play a critical role in the DNA damage response? One important piece of evidence that hints at such a control mechanism is the result of a large-scale mass-spectrometry screen that sought to identify putative ATM and ATR substrates. This screen revealed that a substantial fraction of the putative substrates were proteins known to be involved in RNA metabolism [50]. It is therefore tempting to speculate that the p38MAPK/MK2 complex might function by modulating the translation efficiency of a subset of critical mRNAs, which ultimately dictate cell fate following genotoxic stress. Hopefully future experiments will be able to support or refute this intriguing concept.

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References

1. Abraham RT. Cell cycle checkpoint signaling through the ATM and ATR kinases. *Genes Dev* 2001;15:2177–2196. [PubMed: 11544175]
2. Bartek J, Lukas J. Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer Cell* 2003;3:421–429. [PubMed: 12781359]
3. Harper JW, Elledge SJ. The DNA damage response: ten years after. *Mol Cell* 2007;28:739–745. [PubMed: 18082599]
4. Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. *Nature* 2004;432:316–323. [PubMed: 15549093]
5. Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. *Nat Rev Cancer* 2003;3:155–168. [PubMed: 12612651]
- 6*. Manke IA, Nguyen A, Lim D, Stewart MQ, Elia AE, Yaffe MB. MAPKAP kinase-2 is a cell cycle checkpoint kinase that regulates the G2/M transition and S phase progression in response to UV irradiation. *Mol Cell* 2005;17:37–48. [PubMed: 15629715] This paper demonstrates that MAPKAP Kinase-2 is the critical effector kinase that in the p38MAPK cascade that controls the G1/S and G2/M checkpoints following UV irradiation.
- 7**. Reinhardt HC, Aslanian AS, Lees JA, Yaffe MB. p53-deficient cells rely on ATM- and ATR-mediated checkpoint signaling through the p38MAPK/MK2 pathway for survival after DNA damage. *Cancer Cell* 2007;11:175–189. [PubMed: 17292828] The authors show that the MAPKAP Kinase-2-mediated cell cycle checkpoint is critical for cells lacking a functional p53 response, but not for p53-competent cells, to survive after DNA damage by doxorubicin or cis-platinum. Activation of the p38MAPK/MK2 pathway by these genotoxic agents requires ATR/ATM signaling.
- 8*. Berkovich E, Monnat RJ Jr, Kastan MB. Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair. *Nat Cell Biol* 2007;9:683–690. [PubMed: 17486112] The authors used a 4-OHT-inducible I-PpoI restriction system to induce DSBs at defined locations in the human genome. The authors showed that ATM recruitment to these DSBs required Nbs1, ATM kinase activity and ATM phosphorylation on Ser-1981. ATM and Nbs1 were required for subsequent recruitment of XRCC4 and nucleosome disruption.
9. Downs JA, Nussenzweig MC, Nussenzweig A. Chromatin dynamics and the preservation of genetic information. *Nature* 2007;447:951–958. [PubMed: 17581578]
10. Smerdon MJ, Tlsty TD, Lieberman MW. Distribution of ultraviolet-induced DNA repair synthesis in nuclease sensitive and resistant regions of human chromatin. *Biochemistry* 1978;17:2377–2386. [PubMed: 678515]
11. Fernandez-Capetillo O, Lee A, Nussenzweig M, Nussenzweig A. H2AX: the histone guardian of the genome. *DNA Repair (Amst)* 2004;3:959–967. [PubMed: 15279782]

12. Celeste A, Fernandez-Capetillo O, Kruhlak MJ, Pilch DR, Staudt DW, Lee A, Bonner RF, Bonner WM, Nussenzweig A. Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. *Nat Cell Biol* 2003;5:675–679. [PubMed: 12792649]
13. Celeste A, Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova OA, Reina-San-Martin B, Coppola V, Meffre E, Difilippantonio MJ, et al. Genomic instability in mice lacking histone H2AX. *Science* 2002;296:922–927. [PubMed: 11934988]
- 14**. Falck J, Coates J, Jackson SP. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature* 2005;434:605–611. [PubMed: 15758953]The authors identify a small conserved region in the C-terminus of Nbs1 that is required for ATM recruitment to DSBs, but is not required for binding of the Mre11-Nbs1-rad50 complex to the site. Similar short motifs are identified in ATRIP and Ku80, and are required for the recruitment of ATR and DNA-PKcs, respectively, to sites of DNA damage.
15. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 2003;421:499–506. [PubMed: 12556884]
- 16*. Pellegrini M, Celeste A, Difilippantonio S, Guo R, Wang W, Feigenbaum L, Nussenzweig A. Autophosphorylation at serine 1987 is dispensable for murine Atm activation in vivo. *Nature* 2006;443:222–225. [PubMed: 16906133]The authors demonstrate that transgenic expression of ATM^{TgS1987A} (mutation of Ser to Ala at position 1987, corresponding to human ATM Ser-1981) rescues defective lymphocyte development and genomic instability in ATM^{-/-} mice. Mutation of Ser-1987 to Ala did not abrogate ATM kinase activity and mutant ATM recruitment to damaged DNA was indistinguishable from wildtype ATM.
- 17*. Sun Y, Jiang X, Chen S, Fernandes N, Price BD. A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. *Proc Natl Acad Sci U S A* 2005;102:13182–13187. [PubMed: 16141325]
- 18**. Sun Y, Xu Y, Roy K, Price BD. DNA damage-induced acetylation of lysine 3016 of ATM activates ATM kinase activity. *Mol Cell Biol* 2007;27:8502–8509. [PubMed: 17923702]MY This paper, along with reference 17, shows that acetylation of ATM on Lys-3016 in the FATC domain by Tip60 is the critical first step in ATM activation, autophosphorylation, and dimer dissociation following DNA damage.
- 19**. Gorrini C, Squatrito M, Luise C, Syed N, Perna D, Wark L, Martinato F, Sardella D, Verrecchia A, Bennett S, et al. Tip60 is a haplo-insufficient tumour suppressor required for an oncogene-induced DNA damage response. *Nature* 2007;448:1063–1067. [PubMed: 17728759]The authors demonstrate that Tip60 counteracts myc-induced oncogenesis in an Eμ-myc lymphoma model in a haploinsufficient fashion. Activation of the myc-induced DNA damage response, measured by γH2AX, p-Chk1, p-ATM, p-p53 and p-Bid, was impaired in Tip60 heterozygous Eμ-myc mice, leading to decreased disease-free survival. Examining a series of human lymphomas, head-and-neck and mammary carcinomas, the authors show that monoallelic loss Tip60 is frequently found in these human malignancies.
- 20*. Murr R, Loizou JI, Yang YG, Cuenin C, Li H, Wang ZQ, Herceg Z. Histone acetylation by Trapp-Tip60 modulates loading of repair proteins and repair of DNA double-strand breaks. *Nat Cell Biol* 2006;8:91–99. [PubMed: 16341205]
21. Robert F, Hardy S, Nagy Z, Baldeyron C, Murr R, Dery U, Masson JY, Papadopoulou D, Herceg Z, Tora Ls. The transcriptional histone acetyltransferase cofactor TRRAP associates with the MRN repair complex and plays a role in DNA double-strand break repair. *Mol Cell Biol* 2006;26:402–412. [PubMed: 16382133]
22. Lee MS, Edwards RA, Thede GL, Glover JN. Structure of the BRCT repeat domain of MDC1 and its specificity for the free COOH-terminal end of the gamma-H2AX histone tail. *J Biol Chem* 2005;280:32053–32056. [PubMed: 16049003]
23. Lou Z, Minter-Dykhouse K, Franco S, Gostissa M, Rivera MA, Celeste A, Manis JP, van Deursen J, Nussenzweig A, Paull TT, et al. MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals. *Mol Cell* 2006;21:187–200. [PubMed: 16427009]
24. Stucki M, Clapperton JA, Mohammad D, Yaffe MB, Smerdon SJ, Jackson SP. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. *Cell* 2005;123:1213–1226. [PubMed: 16377563]

- 25**. Mailand N, Bekker-Jensen S, Fastrup H, Melander F, Bartek J, Lukas C, Lukas J. RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell* 2007;131:887–900. [PubMed: 18001824] MY References 25–28 establish that ATM phosphorylation of the adaptor protein MDC1 recruits the E3 ligase RNF8 through its FHA domain to sites of DNA damage. RNF8, together with the E2 Ubc13, generates K63-polyubiquitin chains on histone molecules, leading to ubiquitin-dependent recruitment of 53BP1 and the Brca1-Rap80 DNA repair protein complex. These papers thus connect phosphorylation-dependent signaling by DNA damage-responsive kinases with protein ubiquitination and DNA repair.
- 26**. Kolas NK, Chapman JR, Nakada S, Ylanko J, Chahwan R, Sweeney FD, Panier S, Mendez M, Wildenhain J, Thomson TM, et al. Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. *Science* 2007;318:1637–1640. [PubMed: 18006705]
- 27**. Huen MS, Grant R, Manke I, Minn K, Yu X, Yaffe MB, Chen J. RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. *Cell* 2007;131:901–914. [PubMed: 18001825]
- 28**. Wang B, Elledge SJ. Ubc13/Rnf8 ubiquitin ligases control foci formation of the Rap80/Abraxas/Brca1/Brc36 complex in response to DNA damage. *Proc Natl Acad Sci U S A* 2007;104:20759–20763. [PubMed: 18077395]
- 29*. Dimitrova N, Chen YC, Spector DL, de Lange T. 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. *Nature* 2008;456:524–528. [PubMed: 18931659] This paper shows that 53BP1 is critical for NHEJ-mediated repair of telomeres that were exposed by conditional deletion of TRF2. 53BP1 and ATM were shown to be required for an increase in mobility of deprotected telomeres. The authors propose that 53BP1 binding increase local chromatin mobility to promote NHEJ.
- 30**. Xie A, Hartlerode A, Stucki M, Odate S, Puget N, Kwok A, Nagaraju G, Yan C, Alt FW, Chen J, et al. Distinct roles of chromatin-associated proteins MDC1 and 53BP1 in mammalian double-strand break repair. *Mol Cell* 2007;28:1045–1057. [PubMed: 18158901] The authors show that MDC1 primarily mediates HR repair, in a γ H2AX-dependent fashion that did not require recruitment of 53BP1 or BRCA1. 53BP1 on the other hand, was shown to mediate XRCC4-dependent NHEJ, which was independent of H2AX.
- 31*. Chapman JR, Jackson SP. Phospho-dependent interactions between NBS1 and MDC1 mediate chromatin retention of the MRN complex at sites of DNA damage. *EMBO Rep* 2008;9:795–801. [PubMed: 18583988]
- 32*. Spycher C, Miller ES, Townsend K, Pavic L, Morrice NA, Janscak P, Stewart GS, Stucki M. Constitutive phosphorylation of MDC1 physically links the MRE11-RAD50-NBS1 complex to damaged chromatin. *J Cell Biol* 2008;181:227–240. [PubMed: 18411308]
33. Byun TS, Pacek M, Yee MC, Walter JC, Cimprich KA. Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. *Genes Dev* 2005;19:1040–1052. [PubMed: 15833913]
34. Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* 2003;300:1542–1548. [PubMed: 12791985]
35. Cimprich KA, Cortez D. ATR: an essential regulator of genome integrity. *Nat Rev Mol Cell Biol* 2008;9:616–627. [PubMed: 18594563]
- 36*. Kumagai A, Lee J, Yoo HY, Dunphy WG. TopBP1 activates the ATR-ATRIP complex. *Cell* 2006;124:943–955. [PubMed: 16530042] In this paper the authors demonstrate that TopBP1 is critical for the activation of ATR. Recombinant TopBP1 was shown to increase the kinase activity of Xenopus and human ATR. This activation was dependent on an ATR-activating domain within TopBP1 and mutation of this domain resulted in defective checkpoint activation in Xenopus egg extracts.
- 37**. Jazayeri A, Falck J, Lukas C, Bartek J, Smith GC, Lukas J, Jackson SP. ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. *Nat Cell Biol* 2006;8:37–45. [PubMed: 16327781] The authors show that in response to DSBs, ATR becomes activated in an ATM dependent manner. ATM and Mre11 were required for the resection of DSBs to yield RPA-coated ssDNA, which is critical for the recruitment and activation of ATR/ATRIP complexes. This ATM-dependent activation of the ATR-Chk1 pathway in response to DSBs was restricted to S and G₂ stages of the cell cycle and required CDK activity.

- 38**. Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, Baer R, Lukas J, Jackson SP. Human CtIP promotes DNA end resection. *Nature* 2007;450:509–514. [PubMed: 17965729] In this paper, the authors show that depletion of CtIP increases cellular sensitivity to DSBs. CtIP was recruited to DSBs in S and G₂ stages of the cell-cycle where it interacted with Mre11. CtIP and Mre11 were shown to be required for efficient DSB resection and ATR-Chk1 activation.
39. Schwarz JK, Lovly CM, Piwnica-Worms H. Regulation of the Chk2 protein kinase by oligomerization-mediated cis- and trans-phosphorylation. *Mol Cancer Res* 2003;1:598–609. [PubMed: 12805407]
40. Melchionna R, Chen XB, Blasina A, McGowan CH. Threonine 68 is required for radiation-induced phosphorylation and activation of Cds1. *Nat Cell Biol* 2000;2:762–765. [PubMed: 11025670]
41. Ahn JY, Schwarz JK, Piwnica-Worms H, Canman CE. Threonine 68 phosphorylation by ataxia telangiectasia mutated is required for efficient activation of Chk2 in response to ionizing radiation. *Cancer Res* 2000;60:5934–5936. [PubMed: 11085506]
42. Lee CH, Chung JH. The hCds1 (Chk2)-FHA domain is essential for a chain of phosphorylation events on hCds1 that is induced by ionizing radiation. *J Biol Chem* 2001;276:30537–30541. [PubMed: 11390408]
43. Ahn J, Urist M, Prives C. The Chk2 protein kinase. *DNA Repair (Amst)* 2004;3:1039–1047. [PubMed: 15279791]
44. Gilbert CS, Green CM, Lowndes NF. Budding yeast Rad9 is an ATP-dependent Rad53 activating machine. *Mol Cell* 2001;8:129–136. [PubMed: 11511366]
45. Kumagai A, Dunphy WG. Claspin, a novel protein required for the activation of Chk1 during a DNA replication checkpoint response in *Xenopus* egg extracts. *Mol Cell* 2000;6:839–849. [PubMed: 11090622]
46. Kumagai A, Kim SM, Dunphy WG. Claspin and the activated form of ATR-ATRIP collaborate in the activation of Chk1. *J Biol Chem* 2004;279:49599–49608. [PubMed: 15371427]
- 47*. Liu S, Bekker-Jensen S, Mailand N, Lukas C, Bartek J, Lukas J. Claspin operates downstream of TopBP1 to direct ATR signaling towards Chk1 activation. *Mol Cell Biol* 2006;26:6056–6064. [PubMed: 16880517] The authors show that RNAi-mediated depletion of TopBP1 globally reduced the phosphorylation of known ATR substrates after DNA damage. TopBP1 knockdown did not impair ATR recruitment to the sites of DNA damage. Depletion of claspin, however, specifically prevented ATR-mediated phosphorylation of Chk1, suggesting that claspin operates downstream of TopBP1 to mediate Chk1 activation following DNA damage.
48. Zhao H, Piwnica-Worms H. ATR-mediated checkpoint pathways regulate phosphorylation and activation of human Chk1. *Mol Cell Biol* 2001;21:4129–4139. [PubMed: 11390642]
49. Wang X, Zou L, Lu T, Bao S, Hurov KE, Hittelman WN, Elledge SJ, Li L. Rad17 phosphorylation is required for claspin recruitment and Chk1 activation in response to replication stress. *Mol Cell* 2006;23:331–341. [PubMed: 16885023]
- 50**. Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER 3rd, Hurov KE, Luo J, Bakalarski CE, Zhao Z, Solimini N, Lerenthal Y, et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 2007;316:1160–1166. [PubMed: 17525332] MY This paper describes a mass-spectrometry based proteomic study that combined stable isotope labeling/enrichment with anti-phospho-Ser-Gln antibody immunoprecipitations to identify putative ATM and ATR substrates following DNA damage. The authors identified ~900 sites on 700 proteins, many of which were not previously implicated in the DNA damage response.
51. Donzelli M, Draetta GF. Regulating mammalian checkpoints through Cdc25 inactivation. *EMBO Rep* 2003;4:671–677. [PubMed: 12835754]
52. Jin J, Shirogane T, Xu L, Nalepa G, Qin J, Elledge SJ, Harper JW. SCFbeta-TRCP links Chk1 signaling to degradation of the Cdc25A protein phosphatase. *Genes Dev* 2003;17:3062–3074. [PubMed: 14681206]
53. Ferguson AM, White LS, Donovan PJ, Piwnica-Worms H. Normal cell cycle and checkpoint responses in mice and cells lacking Cdc25B and Cdc25C protein phosphatases. *Mol Cell Biol* 2005;25:2853–2860. [PubMed: 15767688]
54. Falck J, Mailand N, Syljuasen RG, Bartek J, Lukas J. The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. *Nature* 2001;410:842–847. [PubMed: 11298456]

55. Jin J, Ang XL, Ye X, Livingstone M, Harper JW. Differential roles for checkpoint kinases in DNA damage-dependent degradation of the Cdc25A protein phosphatase. *J Biol Chem* 2008;283:19322–19328. [PubMed: 18480045]
- 56*. Kang T, Wei Y, Honaker Y, Yamaguchi H, Appella E, Hung MC, Piwnica-Worms H. GSK-3 beta targets Cdc25A for ubiquitin-mediated proteolysis, and GSK-3 beta inactivation correlates with Cdc25A overproduction in human cancers. *Cancer Cell* 2008;13:36–47. [PubMed: 18167338] In this paper, the authors identify GSK-3 β as a novel kinase that phosphorylates Cdc25A on the known Chk1 site Ser-76, specifically in G₁. Cdc25A phosphorylation by GSK-3 β required a priming phosphorylation, which could be mediated by Plk3. GSK-3 β -mediated phosphorylation promoted ubiquitin-dependent turnover of Cdc25A. In addition, increased levels of Cdc25A and the presence of an inactivating phosphorylation on GSK-3 β were found to be highly correlated in human tumor tissues.
57. Busino L, Donzelli M, Chiesa M, Guardavaccaro D, Ganoth D, Dorrello NV, Hershko A, Pagano M, Draetta GF. Degradation of Cdc25A by beta-TrCP during S phase and in response to DNA damage. *Nature* 2003;426:87–91. [PubMed: 14603323]
58. Sorensen CS, Syljuasen RG, Falck J, Schroeder T, Ronnstrand L, Khanna KK, Zhou BB, Bartek J, Lukas J. Chk1 regulates the S phase checkpoint by coupling the physiological turnover and ionizing radiation-induced accelerated proteolysis of Cdc25A. *Cancer Cell* 2003;3:247–258. [PubMed: 12676583]
59. Zeng Y, Piwnica-Worms H. DNA damage and replication checkpoints in fission yeast require nuclear exclusion of the Cdc25 phosphatase via 14-3-3 binding. *Mol Cell Biol* 1999;19:7410–7419. [PubMed: 10523629]
60. Rothblum-Oviatt CJ, Ryan CE, Piwnica-Worms H. 14-3-3 binding regulates catalytic activity of human Wee1 kinase. *Cell Growth Differ* 2001;12:581–589. [PubMed: 11751453]
61. Lee J, Kumagai A, Dunphy WG. Positive regulation of Wee1 by Chk1 and 14-3-3 proteins. *Mol Biol Cell* 2001;12:551–563. [PubMed: 11251070]
62. O'Connell MJ, Raleigh JM, Verkade HM, Nurse P. Chk1 is a wee1 kinase in the G2 DNA damage checkpoint inhibiting cdc2 by Y15 phosphorylation. *EMBO J* 1997;16:545–554. [PubMed: 9034337]
63. Huang TT, Wuerzberger-Davis SM, Wu ZH, Miyamoto S. Sequential modification of NEMO/IKKgamma by SUMO-1 and ubiquitin mediates NF-kappaB activation by genotoxic stress. *Cell* 2003;115:565–576. [PubMed: 14651848]
64. Saleem A, Datta R, Yuan ZM, Kharbanda S, Kufe D. Involvement of stress-activated protein kinase in the cellular response to 1-beta-D-arabinofuranosylcytosine and other DNA-damaging agents. *Cell Growth Differ* 1995;6:1651–1658. [PubMed: 9019171]
65. Puc J, Keniry M, Li HS, Pandita TK, Choudhury AD, Memeo L, Mansukhani M, Murty VV, Gaciong Z, Meek SE, et al. Lack of PTEN sequesters CHK1 and initiates genetic instability. *Cancer Cell* 2005;7:193–204. [PubMed: 15710331]
- 66**. Wu ZH, Shi Y, Tibbetts RS, Miyamoto S. Molecular linkage between the kinase ATM and NF-kappaB signaling in response to genotoxic stimuli. *Science* 2006;311:1141–1146. [PubMed: 16497931] This paper shows that NEMO, the regulatory subunit of IKK, gets phosphorylated on Ser-85 in an ATM dependent manner following induction of DSBs. Ser-85 phosphorylation on NEMO promotes ubiquitin-dependent nuclear export of NEMO and NEMO-dependent nuclear export of ATM. Cytoplasmic ATM was demonstrated to mediate phosphorylation and activation of IKK.
67. Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev* 2001;81:807–869. [PubMed: 11274345]
68. Obata T, Brown GE, Yaffe MB. MAP kinase pathways activated by stress: the p38 MAPK pathway. *Crit Care Med* 2000;28:N67–77. [PubMed: 10807318]
69. Ben-Levy R, Hooper S, Wilson R, Paterson HF, Marshall CJ. Nuclear export of the stress-activated protein kinase p38 mediated by its substrate MAPKAP kinase-2. *Curr Biol* 1998;8:1049–1057. [PubMed: 9768359]
70. Engel K, Kotlyarov A, Gaestel M. Leptomycin B-sensitive nuclear export of MAPKAP kinase 2 is regulated by phosphorylation. *EMBO J* 1998;17:3363–3371. [PubMed: 9628873]

- 71*. ter Haar E, Prabhakar P, Liu X, Lepre C. Crystal structure of the p38 alpha-MAPKAP kinase 2 heterodimer. *J Biol Chem* 2007;282:9733–9739. [PubMed: 17255097]MY This paper, along with reference 72, describe the X-ray crystal structure of the inactive nuclear p38 α MAPK:MK2 heterodimeric complex, showing that the C-terminal regulatory region of MK2 is sandwiched between the two kinases, which sit face-to-face, burying the MK2-contributed nuclear export signal and masking the catalytic clefts of both kinases away from potential substrates.
- 72*. White A, Pargellis CA, Studts JM, Werneburg BG, Farmer BT 2nd. Molecular basis of MAPK-activated protein kinase 2:p38 assembly. *Proc Natl Acad Sci U S A* 2007;104:6353–6358. [PubMed: 17395714]MY
73. Gaestel M. MAPKAP kinases - MKs - two's company, three's a crowd. *Nat Rev Mol Cell Biol* 2006;7:120–130. [PubMed: 16421520]
- 74*. Raman M, Earnest S, Zhang K, Zhao Y, Cobb MH. TAO kinases mediate activation of p38 in response to DNA damage. *EMBO J* 2007;26:2005–2014. [PubMed: 17396146]The authors show that the p38 MAPKKK TAO is directly phosphorylated and activated by ATM in response to DNA damage, resulting in increased p38MAPK activity. TAO knockdown impaired p38MAPK activation following genotoxic stress and TAO-depleted cells displayed G₂/M checkpoint insufficiency and reduced survival in response to IR.
75. Branchio D, Tanaka N, Jaeschke A, Ventura JJ, Kelkar N, Tanaka Y, Kyuuma M, Takeshita T, Flavell RA, Davis RJ. Mechanism of p38 MAP kinase activation in vivo. *Genes Dev* 2003;17:1969–1978. [PubMed: 12893778]
76. Enslen H, Raingeaud J, Davis RJ. Selective activation of p38 mitogen-activated protein (MAP) kinase isoforms by the MAP kinase kinases MKK3 and MKK6. *J Biol Chem* 1998;273:1741–1748. [PubMed: 9430721]
77. Jiang Y, Gram H, Zhao M, New L, Gu J, Feng L, Di Padova F, Ulevitch RJ, Han J. Characterization of the structure and function of the fourth member of p38 group mitogen-activated protein kinases, p38delta. *J Biol Chem* 1997;272:30122–30128. [PubMed: 9374491]
78. Kotlyarov A, Yannoni Y, Fritz S, Laass K, Telliez JB, Pitman D, Lin LL, Gaestel M. Distinct cellular functions of MK2. *Mol Cell Biol* 2002;22:4827–4835. [PubMed: 12052889]
79. Roux PP, Blenis J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* 2004;68:320–344. [PubMed: 15187187]
80. Hirose Y, Katayama M, Stokoe D, Haas-Kogan DA, Berger MS, Pieper RO. The p38 mitogen-activated protein kinase pathway links the DNA mismatch repair system to the G₂ checkpoint and to resistance to chemotherapeutic DNA-methylating agents. *Mol Cell Biol* 2003;23:8306–8315. [PubMed: 14585987]
81. Mikhailov A, Shinohara M, Rieder CL. Topoisomerase II and histone deacetylase inhibitors delay the G₂/M transition by triggering the p38 MAPK checkpoint pathway. *J Cell Biol* 2004;166:517–526. [PubMed: 15302851]
82. Sun P, Yoshizuka N, New L, Moser BA, Li Y, Liao R, Xie C, Chen J, Deng Q, Yamout M, et al. PRAK is essential for ras-induced senescence and tumor suppression. *Cell* 2007;128:295–308. [PubMed: 17254968]
83. Pedraza-Alva G, Koulis M, Charland C, Thornton T, Clements JL, Schlissel MS, Rincon M. Activation of p38 MAP kinase by DNA double-strand breaks in V(D)J recombination induces a G₂/M cell cycle checkpoint. *Embo J* 2006;25:763–773. [PubMed: 16456545]
84. Kurosu T, Takahashi Y, Fukuda T, Koyama T, Miki T, Miura O. p38 MAP kinase plays a role in G₂ checkpoint activation and inhibits apoptosis of human B cell lymphoma cells treated with etoposide. *Apoptosis* 2005;10:1111–1120. [PubMed: 16151644]
- 85*. Bulavin DV, Higashimoto Y, Popoff IJ, Gaarde WA, Basrur V, Potapova O, Appella E, Fornace AJ Jr. Initiation of a G₂/M checkpoint after ultraviolet radiation requires p38 kinase. *Nature* 2001;411:102–107. [PubMed: 11333986]This paper established that the G₂/M checkpoint following UV irradiation in dermal fibroblasts and HeLa cells requires p38MAPK activity. On the basis of overexpression studies and in vitro experiments, the authors concluded that inhibition/ binding of Cdc25B to 14-3-3 was a likely downstream target for this pathway.
- 86*. Lemaire M, Froment C, Boutros R, Mondesert O, Nebreda AR, Monsarrat B, Ducommun B. CDC25B phosphorylation by p38 and MK-2. *Cell Cycle* 2006;5:1649–1653. [PubMed: 16861915] Using mass spectrometry, the authors confirm that Cdc25B is phosphorylated by MK2 on multiple

sites, including the 14-3-3 binding site Ser-323. In contrast, p38MAPK was shown to phosphorylate Cdc25B on Ser-249.

- 87*. Lopez-Aviles S, Grande M, Gonzalez M, Helgesen AL, Alemany V, Sanchez-Piris M, Bachs O, Millar JB, Aligue R. Inactivation of the Cdc25 phosphatase by the stress-activated Srk1 kinase in fission yeast. *Mol Cell* 2005;17:49–59. [PubMed: 15629716] In *S. pombe*, the authors show that the MK2 homologue Srk1 phosphorylates Cdc25 to induce phosphorylation-dependent binding of Cdc25 to the 14-3-3 homologue Rad24 and subsequent nuclear exclusion. Srk1 activation occurred periodically during G₂, even in unperturbed cells, resulting in delayed mitotic entry. In contrast to mammalian cells, *S. pombe* Srk1 was not critical for cellular survival following UV-induced DNA damage.
88. Lopez-Aviles S, Lambea E, Moldon A, Grande M, Fajardo A, Rodriguez-Gabriel MA, Hidalgo E, Aligue R. Activation of Srk1 by the mitogen-activated protein kinase Sty1/Spc1 precedes its dissociation from the kinase and signals its degradation. *Mol Biol Cell* 2008;19:1670–1679. [PubMed: 18272791]
89. Begley TJ, Rosenbach AS, Ideker T, Samson LD. Hot spots for modulating toxicity identified by genomic phenotyping and localization mapping. *Mol Cell* 2004;16:117–125. [PubMed: 15469827]
90. Huard S, Elder RT, Liang D, Li G, Zhao RY. Human immunodeficiency virus type 1 Vpr induces cell cycle G₂ arrest through Srk1/MK2-mediated phosphorylation of Cdc25. *J Virol* 2008;82:2904–2917. [PubMed: 18160429]
91. Bartz SR, Rogel ME, Emerman M. Human immunodeficiency virus type 1 cell cycle control: Vpr is cytostatic and mediates G₂ accumulation by a mechanism which differs from DNA damage checkpoint control. *J Virol* 1996;70:2324–2331. [PubMed: 8642659]
92. Dahlkvist A, Kanter-Smoler G, Sunnerhagen P. The RCK1 and RCK2 protein kinase genes from *Saccharomyces cerevisiae* suppress cell cycle checkpoint mutations in *Schizosaccharomyces pombe*. *Mol Gen Genet* 1995;246:316–326. [PubMed: 7854316]
93. Perfettini JL, Nardacci R, Bourouba M, Subra F, Gros L, Seror C, Manic G, Rosselli F, Amendola A, Masdehors P, et al. Critical involvement of the ATM-dependent DNA damage response in the apoptotic demise of HIV-1-elicited syncytia. *PLoS ONE* 2008;3:e2458. [PubMed: 18560558]
- 94*. Zeng Y, Sankala H, Zhang X, Graves PR. Phosphorylation of Argonaute 2 at serine-387 facilitates its localization to processing bodies. *Biochem J* 2008;413:429–436. [PubMed: 18476811] In this paper the authors show that MK2 can efficiently phosphorylate Ago2 on Ser-387 and phosphorylation on this site was induced in HEK293T cells overexpressing Ago2 after treatment with arsenite, a known activator of the p38MAPK/MK2 pathway. Mutation of Ser-387 to alanine or pharmacological inhibition of p38MAPK reduced arsenite-induced Ago2 recruitment into P-bodies in H1299 cells.
- 95*. Maitra S, Chou CF, Luber CA, Lee KY, Mann M, Chen CY. The AU-rich element mRNA decay-promoting activity of BRF1 is regulated by mitogen-activated protein kinase-activated protein kinase 2. *RNA* 2008;14:950–959. [PubMed: 18326031] MY Butyrate response factor-1, also known as TIS11B, is critical for the rapid degradation of mRNAs containing AU-rich 3' UTRs. In this paper the authors show that MK2 phosphorylates BRF1 at 3 or more sites to inhibit its mRNA decay-promoting function. Surprisingly, MK2 phosphorylation neither disrupts mRNA binding to BRF1 or recruitment of molecules involved in mRNA degradation to BRF1:mRNA complexes, leaving the mechanism unclear. Reference 96, however, shows that at one of the mapped MK2 sites on BRF1 corresponds to a critical 14-3-3-binding site conserved among TIS/TTP family members. A second MK2 site on BRF1 is an even closer match to the 14-3-3-binding motif, suggesting that 14-3-3-binding to BRF1 likely contributes to the inhibition mechanism.
96. Johnson BA, Stehn JR, Yaffe MB, Blackwell TK. Cytoplasmic localization of tristetraprolin involves 14-3-3-dependent and -independent mechanisms. *J Biol Chem* 2002;277:18029–18036. [PubMed: 11886850]
97. Rousseau S, Morrice N, Pegg M, Campbell DG, Gaestel M, Cohen P. Inhibition of SAPK2a/p38 prevents hnRNP A0 phosphorylation by MAPKAP-K2 and its interaction with cytokine mRNAs. *EMBO J* 2002;21:6505–6514. [PubMed: 12456657]
98. Stoecklin G, Stubbs T, Kedersha N, Wax S, Rigby WF, Blackwell TK, Anderson P. MK2-induced tristetraprolin:14-3-3 complexes prevent stress granule association and ARE-mRNA decay. *EMBO J* 2004;23:1313–1324. [PubMed: 15014438]

Figure 1. Detection of the DSB and recruitment of DNA damage response mediators and effectors

A trimeric complex consisting of Mre11, Rad50 and Nbs1 (MRN) serves as a lesion sensor and recruits the DNA damage checkpoint kinase ATM via a direct interaction between ATM and Nbs1. ATM in turn phosphorylates histone H2AX (yielding so called γ H2AX), creating a phosphoepitope that is engaged by the BRCT domain of MDC1. MDC1 itself is phosphorylated by CK2, which promotes a direct, phospho-dependent interaction between Nbs1 and MDC1. MDC1 is an ATM substrate and this phosphorylation creates a phospho-dependent binding site for the FHA domain of the E3 ubiquitin ligase RNF8. RNF8-mediated ubiquitination of γ H2AX generates a binding site for the ubiquitin interacting motif (UIM) of Rap80 and ubiquitinated γ H2AX. Rap80 serves as a scaffold protein to recruit a DNA repair complex consisting of Abraxas and BRCA1. The interaction between Abraxas and BRCA1 is phospho-dependent and requires the BRCA1 BRCT domains.

Figure 2. Activation of the DNA damage cell cycle checkpoint network results in rapid and persistent Cdk inhibition

Activation of the upstream activating PI3-Kinase-like kinases ATM and ATR triggers signaling through the cell cycle checkpoint signaling cascade. ATM activates the checkpoint effector kinase Chk2 by direct phosphorylation on Thr-68. Activation of ATR requires TopBP1 and ATR is recruited to damaged DNA via its regulatory subunit ATRIP. Efficient ATR-mediated activation of the downstream kinase Chk1 requires the presence of claspin. Both ATM and ATR appear to be required to activate the p38MAPK/MK2 effector kinase complex downstream of TAO kinases in response to DNA damage. The three effector kinases Chk1, Chk2 and MK2 are directly responsible for inhibitory phosphorylations on members of the

Cdc25 family of phosphatases. Additionally, Chk1 phosphorylates and activates Wee1 kinase. Wee1 directly mediates inhibitory phosphorylation of Cdks, which are normally removed through the enzymatic activity of Cdc25 phosphatases to allow progression through the cell cycle.