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Marks to stop the clock: histone modifications and checkpoint regulation in the DNA damage response¹

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Abstract: DNA damage from endogenous and exogenous sources occurs throughout the cell cycle. In response to this damage, cells have developed a series of biochemical responses that allow them to recover from DNA damage and prevent mutations from being passed on to daughter cells. An important part of the DNA damage response is the ability to halt the progression of the cell cycle, allowing damaged DNA to be repaired. The cell cycle can be halted at semi-discrete times, called checkpoints, which occur at critical stages during the cell cycle. Recent work in our laboratory and by others has shown the importance of post-translational histone modifications in the DNA damage response. While many histone modifications have been identified that appear to facilitate repair per se, there have been surprisingly few links between these modifications and DNA damage checkpoints. Here, we review how modifications to histone H2A serine 129 (HSA129) and histone H3 lysine 79 (H3K79) contribute to the stimulation of the G1/S checkpoint. We also discuss recent findings that conflict with the current model of the way methylated H3K79 interacts with the checkpoint adaptor protein Rad9.

Key words: histone modifications, H3K79, H2AS129, checkpoints, DNA damage.

Résume : Les dommages à l’ADN de sources endogènes ou exogènes se produisent tout au long du cycle cellulaire. En réponse à ces dommages, les cellules ont développé une série de réponses biochimiques qui leur permettent de récupérer de ces dommages à l’ADN et qui préviennent que les mutations ne soient transmises aux cellules filles. Une partie importante de la réponse aux dommages à l’ADN est la capacité d’arrêter la progression du cycle cellulaire, permettant au dommage à l’ADN d’être réparé. Le cycle cellulaire peut être arrêté à des moments précis appelés points de contrôle que l’on retrouve à des étapes critiques du cycle cellulaire. Des travaux récents réalisés dans notre laboratoire et ailleurs ont démontré l’importance des modifications post-transductionnelles des histones en réponse aux dommages à l’ADN. Alors que plusieurs modifications d’histones semblent faciliter intrinsèquement la réparation, on a fait étonnamment peu de liens entre ces modifications et les points de contrôle des dommages à l’ADN. Nous passons ici en revue les façons dont les modifications de la sérine 129 de l’histone H2A (H2AS129) et de la lysine 79 de l’histone H3 (H3K79) contribuent à la stimulation du point de contrôle G1/S. Nous discutons également des découvertes récentes qui contredisent le modèle actuel d’interaction de H2K79 et la protéine adaptatrice de point de contrôle Rad9.

Mots-clés : modification d’histones, H3K79, H2AS129, points de contrôle, dommage à l’ADN.

Cell cycle

The processes of cell division are tightly regulated and subject to many controls that can halt progression due to environmental stress. The family of serine/threonine kinases, known as CDKs (cyclin-dependent kinases), is responsible for cell cycle progression. CDKs are inactive until they associate with cyclins. The budding yeast Saccharomyces cerevisiae has only 1 major cell cycle CDK (Cdc28) but a number of different cyclins, the levels of which fluctuate with the cell cycle (recently reviewed in Bloom and Cross 2007). Higher eukaryotes have multiple CDKs, as well as multiple cyclins, that associate in specific combinations to advance the cells through the cell cycle. S. cerevisiae CDK associates with G1-cyclins (Cln1-3) in G1 phase, and this active kinase complex controls progression of the cell cycle by


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activating proteins, such as the Rho-type GTPase Cdc42, which functions in the polarization of the cell and inception of the new bud (Sopko et al. 2007). During all other phases of the cell cycle, CDK function is controlled by the B-type cyclins Clb1-6. Different Clbs promote DNA replication, spindle maturation and segregation, and entry and progression through mitosis (Donaldson 2000; Küntzel et al. 1996; Mendenhall and Hodge 1998).

To ensure that cell cycle activities do not occur prematurely or out of sequence, CDKs and cyclins are specifically regulated. CDK activity is regulated by inhibitory phosphorylation or by association with inhibitory proteins that block ATP-binding sites on the kinases (Mendenhall and Hodge 1998). Cyclins are blocked from associating with CDKs by inhibitory proteins, and while levels of CDKs remain fairly constant, cyclin levels are controlled by rapid degradation via the proteasome (Mendenhall and Hodge 1998). Thus, as the cell progresses through the cell cycle, the levels of different cyclins will fluctuate in a phase-dependent manner. Various feedback mechanisms stimulate the transcription of the subsequent cyclin to advance the cell beyond its current phase. The regulation of the activity of Cdc28/Clb complexes by the inhibitory protein Sic1 illustrates this type of cell cycle control. In response to DNA damage in G1, yeast cells activate Rad53, a checkpoint kinase that causes the downstream suppression of Cln genes, preventing their accumulation. Without the accumulation of Cln1-3, the Sic1 protein, which inhibits the activity of Cdc28/Clb, remains at high levels in the cell, restricting passage from G1 into S phase. Only activation and transcription of the Cln genes can stimulate the phosphorylation of Sic1 and its subsequent ubiquitylation and degradation, releasing the Cdc28/Clb complexes (Harper 2002; Wysocki et al. 2006).

Checkpoints

There are a number of different points during the cell cycle where its progression can be paused to recover from a particular stress, or stopped entirely if the damage caused by the stress cannot be overcome, and these points are collectively referred to as checkpoints. The major checkpoints occur at G1/S (Start), during S phase (intra-S and replication checkpoints), and during G2/M. At any point during the cell cycle, various stresses can cause a cell to arrest, including nutrient deprivation, overcrowding, and DNA damage. Checkpoints are usually regulated by a separate set of sensor proteins and signaling kinases that respond to perceived stresses and send signals to halt the cell cycle. In the case of DNA damage, the detection of lesions or breaks in the DNA requires a cell to repair the damage before progressing to the next phase to ensure that genetic information is not altered or lost (Ataian and Krebs 2006; Bartek and Lukas 2007; Branzei and Foiani 2008; Gontijo et al. 2003; Harrison and Haber 2006; Heideker et al. 2007). The detection of DNA damage is achieved by a number of surveillance proteins that respond to distortions in the DNA double helix, the presence of single-stranded DNA, or unprotected exposed ends of DNA.

Key members of the phosphatidylinositol-3 kinase-like kinase (PI3KK) family of proteins detect and respond to DNA damage, such as double-strand breaks (DSBs), by phosphorylating a wide range of proteins (Cann and Hicks 2007; Lee and Paull 2007). The 2 best characterized of these proteins are Tél1 and Mec1 in S. cerevisiae (ATM and ATR in mammals), which respond to DNA damage by phosphorylating histones (Downs et al. 2000; Du et al. 2006; Fernández-Capetillo et al. 2004; Foster and Downs 2005; Moore et al. 2007), other kinases, adaptor proteins, chromatin modifying proteins, and even themselves (Clerici et al. 2004; Grenon et al. 2006; Morrison et al. 2007; Paciotti et al. 1998; Sweeney et al. 2005); with selection of their targets dependent on what point in the cell cycle the damage occurs. There are a number of mammalian targets of the ATM/ATR kinases, including the well-known oncoprotein p53, the p53-binding protein 53BP1, the BRCA1 oncoprotein, and Smc1. p53 is a transcription factor that initiates transcription of CDK inhibitors to stop cell cycle progression, as well as proapoptotic factors in the presence of a prolonged cell cycle block (Niida and Nakanishi 2006). 53BP1 is related to S. cerevisiae Rad9, and serves in the same capacity as an adaptor protein by stimulating the phosphorylation of the checkpoint effector protein Chk2 (Rad53 in S. cerevisiae) (Pellicioli and Foiani 2005; Sweeney et al. 2005). The oncoprotein BRCA1 has direct activity in cell cycle regulation at the G2/M checkpoint (Gudmundsdottir and Ashworth 2006; Kao et al. 2006; Paull et al. 2000). Smc1, part of the cohesin complex involved in chromosome organization and segregation and DSBR repair, has a well-characterized homolog of the same name in yeast (Watrin and Peters 2006). All of these targets play a role in responding to DNA damage within the context of the cell cycle, preventing cells from progressing without proper repair.

DNA damage response

DNA damage can occur at any stage of the cell cycle and can be caused by both endogenous and exogenous sources. The response to damage depends on the cell cycle stage and the type of damage. DNA can be damaged by ultraviolet light (UV), alkylating agents, and errors introduced by DNA polymerases. These types of damage can be repaired by pathways such as nucleotide excision repair and base excision repair. If these repair events do not occur and cell cycle checkpoints remain active, an alternative response of the cell is to replicate across the lesion, using specialized translesion polymerases, such as Pol ζ. Translesion polymerases are error prone, but do increase the survival of yeast cells by allowing for the completion of DNA replication after exposure to UV and γ radiation and alkylating agents. Pol ζ gene deletion mutants have decreased rates of homologous recombination (HR), indicating that error prone polymerases are also important in other repair pathways (Gan et al. 2008).

Other sources of DNA damage result in the formation of DSBs. DSBs can be induced by ionizing radiation (IR), by conversion of single-strand lesions during replication, or experimentally, using radiomimetic drugs or endonucleases. The pathways of nonhomologous end-joining (NHEJ) and HR, the 2 major pathways responsible for repair of DSBs, have been the most extensively studied mechanisms of
DNA repair with respect to histone modifications, so we will focus on these pathways in this review.

Signaling of DNA damage involves the recognition of lesions or DSBs and the activation of both cell cycle regulators and repair factors. Cell cycle checkpoint factors, such as Chk2 (Rad53), MDC1, and 53BP1, all regulate the progression of the cell cycle to permit the cell time to repair the DNA, which is essential for maintenance of genomic integrity and cell survival. 53PB1, a proposed homolog to the budding yeast Rad9 protein, is an effector protein that stimulates phosphorylation of other checkpoint regulators, such as Chk2 (Pellicioli and Foiani 2005; Sweeney et al. 2005). Chk2, a homolog of the Rad53 protein, is a kinase that plays a role in establishing checkpoints and halting progression through the cell cycle (Nakamura et al. 2004), while MDC1, which currently has no yeast homolog, plays a similar role and is involved in phosphorylation of targets that control cell cycle progression (Stewart et al. 2003). The recruitment of repair factors, which occurs after the detection of DNA damage, is specific for the type of damage, as well as for the state of the cell cycle at the time the damage is detected (Dasika et al. 1999).

When DSBs occur during G1 in haploid cells, the cell can repair the damage through the process of NHEJ, which does not use homologous DNA as a template for the repair process, and can result in deletions or other errors in the DNA sequence following repair (Fig. 1). Yeast usually use NHEJ only at points in the cell cycle where a sister chromatid (or other homologous donor) is not available for HR (Daley et al. 2005; Duda’sová et al. 2004; McGinty et al. 2008; Shrivastav et al. 2008). The repair proteins Ku70 and Ku80, which are abundant in the cell, form heterodimers and bind to both ends of the broken DNA. The Ku dimers then recruit the MRX (Mre11-Rad50-Xbs1) complex of proteins to the break, which may assist in bringing the 2 broken ends together, and also participate in the processing of the ends. The Lig4-Lif1 ligase complex then associates and the 2 ends are ligated together. The process of NHEJ is error prone because it does not use a template to make repairs of the DSB and will, therefore, combine together the free ends of any nearby DNA. In instances where multiple DSBs occur, NHEJ can lead to translocations and to loss of genetic information (Haber 2006).

DSBs that occur in late S phase or in diploid cells can be repaired by HR (Fig. 1). HR has a much lower chance of causing mutations in the genome than NHEJ, but requires that a sister chromatid or other homologous donor be available for use as a template for repair. Sister chromatids are the preferred template for HR even in diploids, because the use of homologous loci can lead to loss of heterozygosity. There are several recent reviews of HR (for example, Ataian and Krebs 2006; San Filippo et al. 2008), so we will only briefly describe this pathway here. HR is a multistep process that includes recognition of the break, processing of the free ends, searching for homology, invasion of the homologous region, replication of the homologous template, and resolution of the Holliday junction. The MRX complex binds to the ends and participates in the 5’ to 3’ resection, forming single-stranded DNA, which is immediately recognized by the single-stranded DNA binding protein replication protein A (RPA). The presence of single-stranded DNA associated with RPA constitutes a key signal for checkpoint activation. After RPA is bound, it recruits Rad52, Rad54, and the Rad55/Rad57 heterodimers. This group of proteins replaces RPA with the strand exchange published by NRC Research Press.
Chromatin

In eukaryotic cells, the DNA must be packaged to fit into the nucleus and to be properly segregated during mitosis and, thus, associates with histones to form nucleosomes, the basic units of chromatin. Two copies each of 4 types of histones (H2A, H2B, H3, and H4) form the core of 1 nucleosome. A total of 146 base pairs of DNA wrap 1 3/4 times around the nucleosome, with different lengths of linker DNA between nucleosomes, forming the 10 nm fiber. Each of the core histone proteins has a core globular region, around which the DNA wraps, and a flexible N-terminal tail (H2A has 2 tails, 1 N-terminal, and 1 C-terminal) rich in basic amino acid residues that associate closely with the negative backbone of the DNA (Chodaparambil et al. 2006; Luger 2003, 2006).

Even at the lowest level of compaction, the 10 nm fiber, DNA in chromatin is relatively inaccessible. The inner surface of the DNA helix wrapped around the nucleosome is hidden from enzymes and other proteins with sequence-specific recognition sites, and the histone tails themselves cover most of the DNA sequence. Modifications to chromatin structure can allow enzymes involved in DNA metabolism access to the DNA. Chromatin can be modified by the physical modification of DNA–histone associations by ATP-dependent chromatin remodeling enzymes, and by post-translational modification of histone tails by enzymes that covalently attach various chemical groups to modifiable amino acids. Some modifications, such as the acetylation of lysine residues or the phosphorylation of serines and threonines, affect the charge of the histone tails, neutralizing the positive charge in the case of lysine acetylation and adding a negative charge in the case of phosphorylation. Other modifications do not change the charge of the tail, but create novel recognition sites on the tails to promote or prevent binding of other proteins. These types of modifications include mono-, di-, or trimethylation of lysines, mono- or dimethylation of arginines, ubiquitination and sumoylation of lysines (Kouzarides 2007; Krebs 2007; Millar and Grunstein 2006; Verdone et al. 2006). Different modifications, and combinations of modifications in a particular sequence, have been shown to be involved in specific nuclear activities, and these sequences of modifications remain relatively well conserved among eukaryotes (Krebs 2007; Millar and Grunstein 2006; Rutherford et al. 2007). For example, methylation of H3K4, which stimulates the acetylation of various other lysines on H3, is linked to transcriptional activation (Wang et al. 2001), while phosphorylation of H2A serine 129 (S129) has been associated with repair of DNA DSBs (Downs et al. 2000; Moore et al. 2007).

While histone modifications have been extensively studied in transcription and repair, only 2 modifications have been strongly connected to checkpoint function. In this review, we will focus on the histone modifications that have been implicated in the management of the cell cycle, particularly in DNA damage checkpoints that halt the cell cycle during the process of DNA repair.

Role of histone H2A phosphorylation

One of the most extensively characterized repair-specific histone modifications is the phosphorylation of histone H2AS129 (H2AX serine-139 in mammals); the phosphorylated form is often designated as γH2A/γH2AX (for recent reviews, see Altaf et al. 2007; Ataian and Krebs 2006; Chambers and Downs 2007; Escargueil et al. 2008; Karagiannis and El-Osta 2007). H2AS129 is phosphorylated by Mec1 and Tel1 kinases, the yeast homologs of the mammalian ATR and ATM kinases, respectively (Downs et al. 2000; Rogakou et al. 1998). These kinases play a number of very important roles in the DNA damage surveillance and repair systems, which are highly conserved among all eukaryotes. γH2AX foci form in regions extending megabases from the site of damage in response to DNA DSBs and UV irradiation in mammals and ~50 kb around breakpoints in yeast (Shroff et al. 2004; Unal et al. 2004).

Mutational analyses of H2AS129 have shown that cells carrying a mutant gene in which S129 has been mutated to an unmodifiable alanine residue (S129A) show sensitivity to DNA DSBs and show an increase in collapsed replication forks (Downs et al. 2000; Moore et al. 2007; Redon et al. 2003, 2006). H2AS129 may also play a role in responding to UV irradiation and oxidative damage, as it is phosphorylated following these treatments, although it is not essential for survival after these types of damage (Moore et al. 2007). The role of γH2A in checkpoint function is controversial, however. In Schizosaccharomyces pombe, lack of γH2A formation results in loss of checkpoint maintenance. Nakamura et al. (2004) showed, in S. pombe, that S129A mutation results in a number of defects, such as loss of IR-induced Crb2 focus formation, and reduced Chk1 and Crb2 phosphorylation. Crb2, the S. pombe homolog of Rad53, is required for the G1 DNA damage checkpoint. They also showed that cells arrested at G1/M (using a temperature-sensitive Cde25 mutant) show dose-dependent checkpoint effects when irradiated with varying levels of IR and then released into the cell cycle. At low levels of IR, H2AS129A mutants exhibit a normal DNA damage checkpoint, similar to wild-type cells, whereas at higher doses of IR, which require longer cell cycle delays for full DNA repair, S129A mutants exit the checkpoint early, and proceed

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through the cell cycle, suggesting that γH2A is more important for the maintenance than the establishment of the checkpoint (Nakamura et al. 2004).

S. cerevisiae, however, appears able to maintain a checkpoint under similar conditions. Downs et al. (2000) showed that in the presence of methyl methane sulphonate (MMS), a DNA alkylating agent known to induce γH2A, synchronized cultures of wild-type and H2AS129A mutants do not show significantly different rates of progression through the cell cycle, as measured by bud index, whether synchronized with nocodazole or α-factor (Downs et al. 2000). However, another study showed that H2AS129A mutants synchronized at G1 enter into S phase more rapidly after IR treatment than wild-type cells, revealing a slight G1 checkpoint defect, and also show a slight affect on intra-S phase checkpoints in synchronized cells treated with MMS (Javaheri et al. 2006). These researchers saw no difference between the mutant and synchronized cells treated with MMS (Javaheri et al. 2006). The association with Rad9 provides the most notable link between γH2A and checkpoint function (Fig. 2). In S. cerevisiae, Rad9 has been shown to interact with γH2A (Hammet et al. 2007), and the same has been shown for the S. pombe homolog Crb2 (Nakamura et al. 2004). Rad9 contains the highly conserved tandem BRCA1 C-terminal (BRCT) protein domains that have been shown to be indispensable for its recruitment to γH2A. These 2 tandem BRCT domains (BRCT2), when tested against peptides specific for the C-terminal tail of H2A and γH2A, show an affinity for only γH2A. Comparing this with the BRCT domains of other proteins, such as mammalian MDC1 and BRCA1, we see that Rad9 is specific for γH2A, while the other BRCT2 proteins do not recognize either H2A or γH2A. This specific recruitment of Rad9 to γH2A has also been associated with its hyperphosphorylation, as well as the hyperphosphorylation of Rad53, the S. cerevisiae homolog to Chk2 (Hammet et al. 2007; Soulier and Lowndes 1999). Rad9 and its homologs in higher eukaryotes will be further discussed in subsequent sections.

While the role of γH2A in checkpoint activation is not entirely clear, dephosphorylation of γH2A appears to have an important role in checkpoint recovery (at least for the G2/M checkpoint) after repair is completed. The yeast Pph5 phosphatase, which dephosphorylates Rad53 (O’Neill et al. 2007), is needed to remove the phosphate from S129 as well (Keogh et al. 2006). This dephosphorylation occurs on γH2A that has been displaced from chromatin. Similar results have been observed for mammalian γH2AX, where protein phosphatase 2A is required to remove γH2AX foci, although defects in protein phosphatase 2A function may prolong the repair itself rather than affect checkpoint recovery (Chowdhury et al. 2005). γH2A turnover has also been linked to checkpoint adaptation, a phenomenon in which cells eventually re-enter the cell cycle, despite the persistence of unrepaired damage (Papamichos-Chronakis et al. 2006).

Role of histone H3 methylation

Another modification linked to checkpoint function in response to DNA damage is methylation of H3K79, although, like H2AS129, its role has been somewhat controversial. Methylation of H3K79 (H3K79me) is a constitutive process, and appears to occur after nucleosomes are assembled. The level of monomethylated H3K79 in HeLa cells decreases in S phase, possibly due to the dilution of the original histone proteins during DNA replication, reaching the lowest levels during G2, increasing during mitosis, and finally reaching the highest levels in G1 (Feng et al. 2002). Levels of dimethylated H3K79 in S. cerevisiae have been shown to be constant throughout the cell cycle and during DNA damage (Wysoki et al. 2005). Feng et al. (2002) proposed that the decrease in monomethylation during S phase was not likely a result of dilution, as decreases of similarly constitutive modifications are not observed during S phase. One possible explanation for this discrepancy is that many modified histone residues are located on the tails, making them accessible for the quick addition of marks before or after nucleosome assembly. H3K79 is hidden in the nucleosome core, making it difficult for histone-modifying enzymes to make modifications immediately after assembly has occurred (Luger et al. 1997). This inaccessibility would require chromatin remodelers to expose the residue for the histone methyltransferases, explaining why methylation of K79 occurs so slowly following DNA replication (Fig. 2).

As described above, when damage occurs in G1, Ku70 and Ku80 bind to the ends of the break, initiating NHEJ. The MRX complex then binds to the break, initiating the processing of DNA ends; in NHEJ, single-strand resection is prevented and, instead, blunt-ended DNA, a substrate for DNA ligase, is ultimately generated. The presence of MRX bound to DNA is a checkpoint activation signal. The kinases Tel1 and Mec1 (with their respective partners Tel2 and Ddc2) associate with the MRX complex, leading to the recruitment of Mec1 and Tel1 to the area of the break, resulting in the phosphorylation of H2AS129 both up- and downstream of the break (Anderson et al. 2008; Takai et al. 2007). The SWR1 and INO80 ATP-dependent chromatin remodelers and the NuA4 acetyltransferase are next recruited to the lesion (Downs et al. 2004; Morrison and Shen 2005; Van Attikum et al. 2007). It has been proposed that the action of these remodelers exposes the constitutive H3K79me, thus allowing the adaptor protein Rad9 to bind. The binding of Rad9 leads to its activation, activation of Rad53 by Mec1 and, thus, activation of the G1/S checkpoint (Fig. 2). Recent findings challenge this proposed model by showing that...
some of the chromatin remodelers recruited to the area are not involved in exposing H3K79 (Javaheri et al. 2006), and that Rad9 does not actually bind to methylated K79 (Lancelot et al. 2007). Here, we discuss the conflicting results.

Feng et al. (2002) first discovered the methyltransferase required for H3K79 methylation using a polyclonal antibody that recognizes only monomethylated H3K79. They identified Disruptor of Telomeric silencing 1 (Dot1, also known as Kmt4) as the enzyme responsible for methylation of H3K79, and found that it was conserved from yeast to humans. Dot1 is not only responsible for monomethylation of H3K79, but also dimethylation and trimethylation; these higher methylation states depend on H2BK123 ubiquitylation (K120 in humans) in a histone cross-talk pathway (Feng et al. 2002; McGinty et al. 2008; Ng et al. 2002; Shahbazian et al. 2005; Van Leeuwen et al. 2002; Wysocki et al. 2005). Further evidence supporting histone dilution as the reason for the decrease in H3K79me during S phase comes from in vitro experiments showing that fully assembled nucleosomes are required for Dot1 to add the methyl group onto H3K79; this requirement shows that the modification must be made after nucleosomes are assembled (Feng et al. 2002).

Rad9 and the human homolog 53BP1 have both been proposed to bind to H3K79me, which is supported by pulldown assays using the tandem Tudor domain of 53BP1 (residues...
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break (Lazzaro et al. 2008). Mediating this effect on the model is the fact that this latter experiment was performed in G2-synchronized cells, a time when HR is the primary form of DSB repair, and when dot1 and rad9 mutant cells do not show any checkpoint defect (Grenon et al. 2007).

The role of H3K79me in repair is still unresolved. We believe the most compelling evidence still supports a model such as that illustrated in Fig. 2, in which exposure of a normally buried modification results in recruitment or stabilization of Rad9 binding to chromatin. γH2A binding and DNA binding could independently or additively contribute to Rad9 recruitment, or to its subsequent activity.

Conclusions: Rad9 and beyond

Rad9 homologs in higher eukaryotes have been difficult to identify, owing to lack of strict sequence homology. This lack of sequence homology becomes less troublesome when the secondary structures of the conserved protein domains are compared with proteins with similar checkpoint activities in other species. The mammalian checkpoint protein p53-binding protein 1 (53BP1), BRCA1, and MDC1 have been shown to possess BRCT2 domains and tandem Tudor (Tudor2) domains that interact with H3K79me. The BRCT2 domain of 53BP1 is structurally the most similar to Rad9, despite the lack of sequence identity. Yeast lack homologs of BRCA1 or MDC1. While 53BP1, BRCA1, and MDC1 all have roles in cell cycle checkpoints and genome maintenance, 53BP1, like Rad9, specifically associates with γH2A, as well as H3K79me, and also facilitates phosphorylation of Chk2, the mammalian Rad53 homolog (Huyen et al. 2004; Javaheri et al. 2006; Lancelot et al. 2007; Nakamura et al. 2004; Sweeney et al. 2005). Phosphorylation of Chk2, or Rad53, activates the kinase activity of Chk2/Rad53. Chk2/Rad53 have a number of phosphorylation targets by which they regulate the progression of the cell cycle or activate cell cycle checkpoints.

In summary, stimulation of a checkpoint response in the presence of DNA damage during G1 cells is facilitated by 2 critical histone marks. One is the constitutive methylation of H3K79, and the second is the damage-induced phosphorylation of H2AS129. (H3K79 di- and trimethylation is further controlled by a third critical mark, H2BK123 ubiquitylation.) Neither of these marks alone is enough to efficiently recruit Rad9 to chromatin, and without these marks the activating phosphorylation of Rad9 is reduced, affecting the downstream role of Rad9 in checkpoint activation while DNA repair proceeds. The recruitment of Rad9 to these histone modifications connects the DNA damage checkpoint response to the epigenetic changes that occur during DNA repair, and specifically link the checkpoint to modifications that have been shown to be important for the repair process itself, in addition to their checkpoint functions.

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