Cell Cycle

ISSN: 1538-4101 (Print) 1551-4005 (Online) Journal homepage: https://www.tandfonline.com/loi/kccy20

Paving the way for H2AX phosphorylation: Chromatin changes in the DNA damage response

Nabieh Ayoub, Anand D. Jeyasekharan, Juan A. Bernal & Ashok R. Venkitaraman

To cite this article: Nabieh Ayoub, Anand D. Jeyasekharan, Juan A. Bernal & Ashok R. Venkitaraman (2009) Paving the way for H2AX phosphorylation: Chromatin changes in the DNA damage response, Cell Cycle, 8:10, 1494-1500, DOI: 10.4161/cc.8.10.8501

To link to this article: https://doi.org/10.4161/cc.8.10.8501

[Cell Cycle 8:10, 1494-1500; 15 May 2009]; ©2009 Landes Bioscience

Extra View

Paving the way for H2AX phosphorylation

Chromatin changes in the DNA damage response

Nabieh Ayoub, Anand D. Jeyasekharan, Juan A. Bernal and Ashok R. Venkitaraman*

The Medical Research Council Cancer Cell Unit; Cambridge, UK

Key words: heterochromatin, euchromatin, HP1β, casein kinase 2 (CK2), DNA damage, H2AX phosphorylation, FRAP, FLIP, 2D gels

The dynamics of chromatin-associated proteins control the accessibility of DNA to essential biological transactions like transcription, replication, recombination and repair. Here, we briefly outline what is known about the chromatin changes that occur during the cellular response to DNA breakage, focusing on our recent findings revealing that the chromatin factor HP1β is mobilized within seconds after DNA damage by an unrecognized signaling cascade mediated by casein kinase 2 (CK2) phosphorylation, paving the way for histone H2AX phosphorylation. We also show here that HP1β mobilization is neither associated with histone H3 modification on Ser10, an alteration proposed to assist in HP1 ejection from chromatin, nor with evidence of a physical interaction between HP1β and the CK2 regulatory subunit. Interestingly, following its rapid mobilization, we find that HP1β gradually re-accumulates on damaged chromatin over a longer time period, suggesting that temporal changes in HP1β dynamics and interaction with chromatin may assist in different stages of the cellular response to DNA breakage.

Chromatin Packages the Eukaryal Genome and Regulates its Function

Eukaryotic DNA is organised into a higher-order structure, called chromatin, which was first identified through its affinity to basophilic dyes by Walther Flemming in 1882.[1] Staining the nucleus with DNA dyes revealed two main types of chromatin: a lightly stained euchromatin or a densely stained heterochromatin, which respectively were thought for many years to correspond to a decondensed, transcriptionally-active structure or a highly condensed, non-transcribed one.[2,3] Heterochromatin; in turn, is either constitutive, remaining condensed through all stages of the cell cycle (e.g., at centromeric regions) or facultative, capable of periodic reactivation in certain cell types, or at different stages in their division (e.g., inactive X-chromosome).[4-7] Distinct histone variants and post-translational modifications of histone proteins play an essential role in the establishment, maintenance and inheritance of each type of chromatin structure during cell division.[8-11]

Chromatin serves two main functions. The first is structural; chromatin enables more than two meters of DNA to be packaged into a eukaryotic nucleus with the diameter of a few microns. Around 146 bp of DNA is wrapped around an octameric core of histones, comprising two copies each of the four histones H2A, H2B, H3 and H4, to form a nucleosome—the basic subunit of chromatin. Nucleosomes are connected by DNA linkers to form a poly-nucleosomal thread which is then folded into a 30 nm fibre. Overlayed on this basic organization, histone variants and non-histone proteins like the heterochromatin protein 1 (HP1, also called CBX, Chromobox Homolog[12]) or polycomb proteins help to fold chromatin into higher-order structures.[5,6,9,13]

Chromatin's second role is to regulate genome function and duplication. Thus, chromatin structure controls the execution of various DNA-related transactions such as DNA replication, transcription, recombination and repair.[8,9] Consequently, abnormalities in chromatin structure have been linked to several human diseases including cancer.[14-16]

Chromatin Plasticity via Alterations in the Histone Code

To serve these functions, chromatin structure is dynamically regulated during DNA transcription, replication, or repair, to generate distinct states permissive for these different transactions to take place.[8,9,17] Broadly speaking, alterations in chromatin structure can be achieved via three different routes.[11] Histone chaperones promote the incorporation of specific histone variants during processes like replication, transcription, repair or condensation, which fulfil distinct functions. For example, the histone chaperone, chromatin assembly factor 1 (CAF-1), promotes the loading of histone H3.1 variants at sites of UV-induced damage to mark them for repair.[11] Chromatin re-modelers disrupt histone-DNA interactions in an ATP-dependent manner, and are implicated in transcriptional regulation, as well as DNA damage repair. The multi-subunit complex SWI/SNF for instance, can mobilise nucleosomes across DNA or facilitate the removal of

*Correspondence to: Ashok R. Venkitaraman; Hutchison/MRC Research Centre; Hills Road; Cambridge CB2 0XZ UK; Email: arv22@cam.ac.uk

Submitted: 03/18/09; Accepted: 03/20/09

Previously published online as a *Cell Cycle* E-publication: http://www.landesbioscience.com/journals/cc/article/8501

the H2A-H2B dimer from the nucleosome.[18,19] Finally, a histone code specifies different chromatin states. The code, first defined by David C. Allis,[8] is now believed to comprise at least eight distinct types of post-translational modification (PTM; e.g., methylation, acetylation, etc.,) to specific residues in the tails of histones like H3 or H4.[8,20] The code is, in part, translated via non-histone proteins—termed effectors—which contain domains that bind different histone-tail modifications, and are recruited through them to specific regions of chromatin.[21]

The permutation and combination of different histone-tail modifications, with the proteins that recognize them, enables the specification of many distinct, finely-tuned, chromatin states. Several features are relevant to the repertoire of states that can be specified. First, chromatin states tend to be determined by a pattern of histone-tail modifications rather than a single one.[8] For instance, similar histone modifications on different residues can induce either relaxation or condensation of chromatin. Tri-methylation of Lys9 in the tail of histone H3 (i.e., H3K9) or H4K20 is abundant in pericentric constitutive heterochromatin,[22,23] whereas tri-methyl H3K27 is enriched in the facultative inactive X chromosome.[24] Conversely, tri-methylation of H3K4 or acetylation of H3K9 are both associated with active gene expression in euchromatic regions.[25-27] Second, several distinct domains occur in different effector proteins that are capable of binding histone-tail modifications. Thus, acetylated lysine residues generate specific binding sites for different proteins containing 'bromodomains', like CBP, Gcn5, PCAF, TAF1 or RSC4.[28-32] For example, RSC4, a component of the chromatin-remodeling complex RSC binds through its bromodomain to acetylated H3-K14 to promote gene activation,[33,34] while methylation of H3K9 and H3K27 provides binding sites for the chromodomains of HP1 and Polycomb (PC) proteins.[35-40] Finally, it should be noted that alterations in the histone code may also directly modulate chromatin structure. For example, H4K16 acetylation, a hallmark of transcriptionally active chromatin, restrains the folding of 30 nm fiber into higher-order structures.[41-43]

Interestingly, there is evidence to suggest that there is a fourth route for the plasticity of chromatin structure. Alongside changes in the histone code, a so-called sub-code of PTMs that occur on histone-binding effector proteins has recently been proposed to play a role in modulating chromatin structure. For example, HP1γ phosphorylation can modulate the transcriptional silencing activity of chromatin in experimental conditions;[44] however, whether such modifications play a physiologically relevant role has been unclear. An example arising from our work[45] that links sub-code alterations to changes in chromatin structure during the response to DNA breakage will be discussed here.

Chromatin Alterations in the DNA Damage Response

We will begin with a brief summary of known chromatin alterations in the DNA damage response. It is clear from much recent work that proteins which mediate the sensing, signaling and repair of DNA lesions are rapidly recruited to sites of DNA damage and this recruitment is accompanied by changes in chromatin structure.[9] Teleologically speaking, relaxation of the chromatin structure surrounding damage sites could facilitate the recruitment of the cellular machinery that mediates the DNA damage response. Consistent with this notion, chromatin relaxation following different types of DNA damage has been recently reported.[46-48] Interestingly, it may be triggered by signals that depend on PIK-family protein kinases like ATM which are currently believed to be the most proximal elements of the DNA damage response,[48] or it may be independent of them.[46,47] Thus, local heterochromatin relaxation after the exposure of cells to ionising radiation is mediated by ATM phosphorylation of the KAP-1 protein,[49] while H2B mobilisation after laser-induced DNA breakage occurs in an ATM-independent manner.[46]

Notably, changes in the histone code are proximal events in the DNA damage response cascade. Indeed, H2AX phosphorylation, one of the most-studied changes in the histone code, occurs within minutes at the sites of DNA damage and is considered to be the earliest known marker of DNA damage.[50-52] It is followed be an increase in the acetylation of H3 at K9, K14, K18, K23 and K27 and of H4 at K5, K8, K12 and K16.[53,54] Acetylation of lysine residues neutralizes the positive charges on histones and reduces the affinity between histones and DNA, a plausible route to relaxation of the normally tight chromatin structure. Thus, these changes are expected to facilitate the access of different DNA repair proteins to the sites of damage.[54] Indeed, the recruitment of DNA repair factors to ionizing radiation-induced foci is impaired in H2AX null cells.[55] Consequently, these cells are chromosomally unstable and show an increased susceptibility to cancer.[56,57]

The precise mechanism of how DNA damage is sensed and how this leads to the propagation of H2AX phosphorylation remains unknown. Several lines of evidence suggest that both alterations in the structure and dynamics of chromatin are involved in the sensing and initiation of the DNA damage response. Exposure of cells to hypotonic stress, which is expected to trigger changes to the chromatin structure, has been shown to activate ATM kinase.[58] The induction of H2AX phosphorylation following damage was inhibited in cells depleted of the SWI/SNF chromatin remodeler complex whereas ATM autophosphorylation and its recruitment to chromatin after DNA damage remains intact.[59] This suggests that nucleosome mobilisation may play a role in ATM-dependent phosphorylation of H2AX. Intriguingly, prolonged tethering of DNA repair proteins like NBS1, MRE11, MDC1 and ATM to chromatin suffices to activate H2AX phosphorylation, even in the absence of DNA damage.[60] Also, it has been recently shown that the chromatin remodeler complex, Tip60, catalyses the acetylation-dependent ubiquitination of H2AX variant which facilitates its removal from the nucleosome after DNA damage.[61,62] Together, these findings indicate that damage-induced chromatin alterations are an important component of the cellular response to genotoxic lesions, but provide little insight into how they may work.

In this review, we will discuss our recent demonstration that dynamic mobilisation of the chromatin factor HP1β is a proximal event in the sensing and signaling of DNA breakage. We find that HP1β mobilisation is triggered by the damage-induced modification of a single Thr residue, Thr51, by a mechanism that depends on the kinase CK2. Suppression of HP1β mobilisation diminishes

Hs-HP1β	42-WKGFSDEDNTWEPEENLDCP-61
Mus-HP1β	42-WKGFSDEDNTWEPEENLDCP-61
Hs-HP1α	41-WKGFSEEHNTWEPEKNLDCP-60
Mus-HP1α	41-WKGFSEEHNTWEPEKNLDCP-60
Hs-HP1γ	51-WKGFTDADNTWEPEENLDCP-70
Mus-HP1γ	41-WKGFTDADNTWEPEENLDCP-60
Gallus-HP1	41-WKGFSDEDNTWEPEENLDCP-60
Dros-HP1	45-WKGYPETENTWEPENNLDCP-63

Figure 1. Thr51 is evolutionarily conserved. An alignment of the conserved residues surrounding Thr51 in human (Hs) HP1α, β and γ isoforms, and in their orthologues from mouse (Mus), chicken (Gallus) and Drosophila (Dros), is shown.

H2AX phosphorylation, thus far the earliest known event in signaling DNA breakage. Here, we describe our findings and their implications.

HP1 Dynamics Link Chromatin Structure to the DNA Damage Response

Heterochromatin protein 1, an evolutionary conserved protein from yeast to humans, was originally identified as a major component of heterochromatin.[63,64] Three distinct mammalian isoforms of HP1 have been identified HP1α, HP1β and HP1γ. All variants are abundant in centromeric heterochromatic foci, but HP1β, and in particular, HP1γ, also localize to euchromatic regions. Localisation of HP1 protein to chromatin is mediated by its ability to interact with DNA and histones.[12,65] One binding site of particular interest is methylated H3K9 to which HP1 binds directly through its chromodomain.[35,36]

Surprisingly, HP1 proteins can either facilitate or suppress the transcriptional activity of certain genes. These seemingly contradicting functions of HP1 are mediated through its various interacting protein partners. Indeed, HP1 proteins can interact with, and recruit, repressor proteins such as KAP-1,[66] DNA and histone methyltransferases.[67,68] On the other hand, Swi6 protein (the sole HP1 homologue in *S. pombe)* recruits Epe1,[69] an anti-silencing protein that enhances RNA pol-II accessibility and transcription within heterochromatic regions.[70]

Given that HP1 is an abundant chromatin protein, it has recently been used as a marker to track changes in chromatin dynamics.[71-73] Notably, dynamic studies of green fluorescent protein (GFP) tagged HP1 in living cells reveal that HP1 has different residence times on heterochromatin and euchromatin, possibly accounting for the dynamic behaviour of chromatin. The exchange rate of HP1 protein within heterochromatic foci is about 10 times slower compared to euchromatic regions.[71,72] This differential mobility of HP1 protein on chromatin might be involved in sensing and activating the DNA damage response. In line with this, it has been recently shown that the spreading of H2AX phosphorylation around a double-strand break is not continuous, as it skips over heterochromatic regions marked by HP1α foci.[74,75] These observations imply that certain epigenetic features of the heterochromatin structure, modulated by the

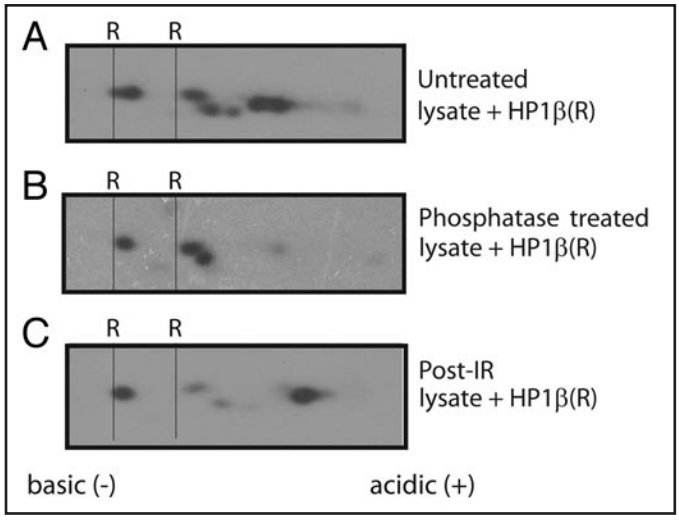

Figure 2. Phosphorylation of HP1β detected by 2D gel electrophoresis. HeLa nuclear extracts were resolved by 2D gel electrophoresis using IEF on a pH 4.5–5.5 gradient in the first (horizontal) dimension, and 12% SDS-PAGE in the second (vertical) dimension. HP1β was detected by western blotting with a specific monoclonal antibody (Abcam). Acidic and basic ends of the horizontal gel are marked. Samples were (A) lysates from undamaged cells; (B) lysates from undamaged cells, treated with λ phosphatase; or (C) lysates prepared from cells 5 min after their exposure to 10 Gy of X-rays. 'R' marks the migration of recombinant HP1β purified from *E. coli* as a reference. The recombinant protein is resolved into two spots with relatively basic isoelectric points in the first dimension, consistent with the calculated pI (4.86) of the unmodified recombinant protein, and suggestive of the generation of two products by N-terminal clipping following thrombin digestion during cleavage from GST.

HP1 proteins, prevent the activation of H2AX phosphorylation after DNA damage. Indeed, our results reveal that DNA damage induces HP1β mobilisation and dispersal from DNA damage sites. This dispersal is mediated by phosphorylation of Thr51 in a CK2-dependent manner. Interestingly, suppression of HP1β mobilisation either by inhibiting CK2 kinase activity or by fusing H2B to HP1β, decreases H2AX phosphorylation after DNA damage. Thus, our work provides a first link between the dynamics of HP1β and the DNA damage response.[45]

The relationship between alterations in HP1β dynamic behaviour and the mechanisms that modulate H2AX modification are not yet clear. One possibility is that the phosphorylation of Thr51 in HP1β, and the consequent mobilization of HP1β from chromatin, may provide a trigger for ATM activation. However, chemical inhibition of the PIK kinases can moderately diminish Thr51 modification (see Fig. S10 in ref. 45), suggesting that there is cross-talk between the pathways, rather than a strictly linear hierarchy of events. For example, HP1β mobilisation may in some way enhance the activity of PIKKs in the vicinity of DNA breaks (e.g., by facilitating PIKK recruitment to damage sites, or improving their access to substrates). Enhanced PIKK activity could, in turn, promote HP1β modification, as discussed later in this article. These possibilities are not mutually exclusive, and further work will be required to address the relationship between HP1β dispersal and PIKK activity.

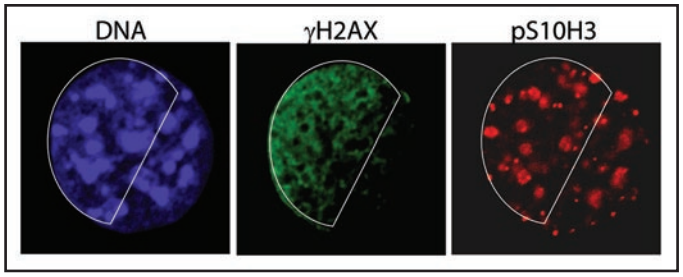

Figure 3. DNA damage does not induce changes in H3Ser10 phosphorylation. Laser-guided DNA damage was induced using 405 nm laser light in the areas bounded by the white lines in living MEFs sensitized by a 5 min exposure to Hoechst 33342, as previously described.[45] Ten min afterwards, cells were fixed and stained for DNA (blue), γH2AX (green) and phosphorylated H3Ser10 (red).

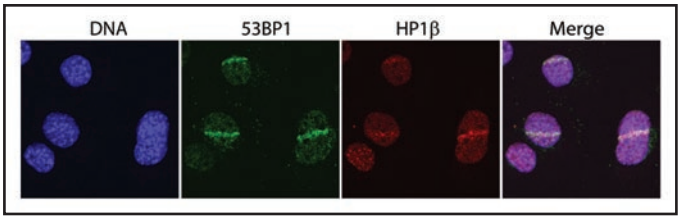

Figure 4. Gradual re-accumulation of HP1β after dispersal by DNA damage. Targeted DNA damage was induced in living MEFs sensitized by a 5 min exposure to Hoechst 33342 as previously described.[45] One hour afterwards, cells were fixed and stained for DNA (blue), 53BP1 (green) and HP1β (red). A merged image is depicted in the final column. Although it is rapidly dispersed within seconds after DNA damage,[45] these images show that endogenous HP1β gradually re-accumulates at damage sites by 1 hr after its initial dispersal.

The evolutionary conservation of Thr51, from Drosophila, to all three mammalian HP1 isoforms (Fig. 1), and its position within the chromodomain, together raises the possibility that its modification represents an important avenue through which HP1 dynamics and localisation can be regulated. Although we have demonstrated that Thr51 phosphorylation is sufficient to disrupt the binding between the HP1β chromodomain and the methylated peptide of H3 in vitro, it is not yet clear that this modification alone suffices to release HP1 from chromatin in vivo. On the one hand, overexpression of HP1β and HP1α in which Thr51 is mutated to either Ala or Glu is sufficient to de-localize the proteins from chromatin. On the other hand, we find using 2D gel electrophoresis that HP1β is phosphorylated at several sites, and that this phosphorylation pattern changes after DNA damage (Fig. 2). Thus, it remains possible that these additional HP1 modifications may help to regulate its dynamics before, as well as after, DNA damage.

Changes in the histone code have been proposed to aid in the dispersal of HP1 from chromatin. For instance, it has recently been shown that phosphorylation at Ser10 of H3 mediates HP1 ejection from chromatin at entry into mitosis.[76] We have therefore tested whether H3 modification on Ser10 may also play a role in HP1β dispersal after DNA damage. Following laser-induced DNA damage using the methods we have reported,[45] cells were probed for both H3 Ser10 and H2AX phosphorylation by immunostaining. Changes in neither the distribution nor the abundance of Ser10 phosphorylation between the damaged and undamaged half of the nucleus were observed (Fig. 3) suggesting that histone H3 phosphorylation on Ser10 is unlikely to contribute to damage-induced alterations in HP1 dynamics.

We find that the rapid dispersal of HP1β protein from chromatin following DNA damage is a transient event; HP1β re-accumulates by around 60–90 min afterwards.[45] This may simply reflect the completion of repair, but alternatively and additionally, may also reflect a requirement for other HP1 functions, such as the suppression of transcriptional activities at damage sites. It has been recently shown that transcriptional activity is suppressed 1 hr after irradiation at sites positive for H2AX phosphorylation,[77] a time at which we find that HP1β reaccumulates both on euchromatin and heterochromatin (Fig. 4). The correlation between these events raises the possibility that HP1β re-accumulation may help to inhibit illegitimate transcriptional activity from a damaged DNA template undergoing repair. Moreover, methylated H3K9 remains intact at the site of damage (see Fig. S3, ref. 45), which might serve as a constitutive repressive mark required for the subsequent re-accumulation of HP1β protein at the damage sites.

CK2 Kinase in the DNA Damage Response

Casein kinase 2 (CK2) is a ubiquitously expressed kinase, which has two catalytic subunits α and α', in addition to a regulatory β subunit.[78] CK2 is known to phosphorylate Ser/Thr residues and is involved in various cellular processes lincluding proliferation, differentiation, apoptosis, tumorigenesis or DNA repair.[79] Interestingly, CK2 constitutively phosphorylates certain proteins involved in the DNA repair pathway such as XRCC1,[80] and MDC1.[81-84] On the other hand, CK2 inducibly modifies other substrates after DNA damage.[85] In yeast, Ser1 of H4 is phosphorylated by CK2 in response to double-strand breaks.[85] In mammalian cells, we find that DNA damage triggers phosphorylation of HP1β at Thr51 by CK2.[45] Interestingly, evidence that CK2 phosphorylation can modulate HP1 function has also been reported in Drosophila and yeast, where this modification influences HP1-mediated transcriptional silencing.[86-88]

It is still unclear how CK2 can be activated and targeted to phosphorylate HP1β specifically at sites of DNA breakage. One possibility is that CK2 is recruited to these sites. Alternatively, DNA damage might induce the interaction of CK2 with HP1, even without detectable co-localization. Thus far, our data do not support either possibility. We cannot detect a damage-induced interaction between HP1β protein and CK2 by immunopprecipitation (Fig. 5A and B). Recruitment of neither the α' nor the β subunit of CK2 to damage sites can be detected by immunofluorescence (Fig. 5C).

Moreover, the relationship between PIKK activity and CK2 activation by DNA damage is yet to be thoroughly explored. As noted before, although HP1β appears to be directly modified by CK2,[45,87,88] but not the PIKK enzymes,[89] we find that chemical inhibition of PIKKs can nevertheless moderately diminish the phosphorylation of HP1β Thr51 after DNA damage. We have

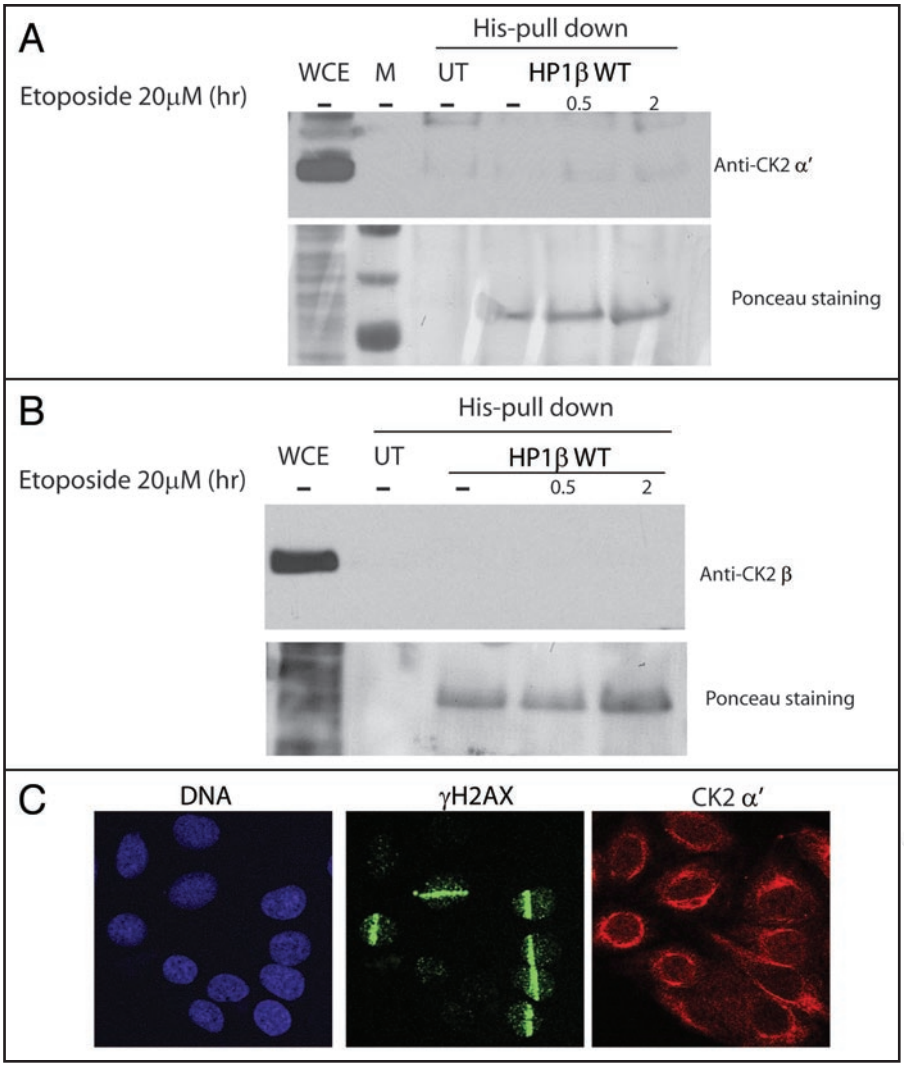

Figure 5. CK2 neither co-immunoprecipitates with HP1β nor co-localizes with it after DNA damage. (A and B) show a western blot analysis of proteins pulled down with His-tagged HP1β transfected in U2OS cells before and after DNA damage induced by etoposide. The blots were probed with antibodies against CK2α′ (A, top), CK2β (B, top). Ponceau staining shows the amount of His-HP1β pulled down and serves as a loading control (A and B, bottom). UT, untransfected cells; M, protein molecular weight markers. (C) laser micro-irradiation with Hoechst pretreatment and subsequent immunofluorescence 10 min post-damage also fails to detect a local enrichment of the CK2 catalytic subunit (red) at sites marked by γH2AX (green). Robust phospho-Thr51 HP1 staining is observed at a similar time point (Fig. S7; ref. 45).

earlier in this article raised the possibility that HP1β modification might facilitate PIKK activation at sites of DNA breakage, and conversely, that PIKK activation could promote HP1β phosphorylation, triggering a local, looping cascade of events to signal DNA breakage. The effect of PIKK enzymes on HP1β phosphorylation seems likely to be indirect, since HP1β neither contains the consensus phosphosite motif, nor is it represented in proteomic screens for PIKK substrates. It is therefore conceivable that PIKKs work to promote CK2 activation at damage sites, directly or indirectly. For example, PIKK enzymes could directly modify CK2, enhancing its activity and ability to modify Thr51 on HP1β. Alternatively, working indirectly, PIKK enzymes might modify and inactivate phosphatases in the vicinity of damage sites, fostering the extent and persistence of Thr51 phosphorylation at these sites. Scenarios of this kind may serve as working hypotheses to help reconcile the alleviation by HP1 depletion of the ATM requirement to signal the presence of heterochromatic DNA breaks,[49] with the moderate suppression of Thr51 phosphorylation by chemical inhibition of PIKKs.[45]

Concluding Remarks

Our findings implicate a transient alteration in chromatin structure mediated by the mobilisation of HP1β as a proximal and important step in the cellular response to DNA breaks, which may promote subsequent events leading to the sensing, signaling and repair of these genotoxic lesions. Indeed, it has previously been shown that cells overexpressing HP1 proteins exhibit sensitivity to ionising radiation,[90] speaking to a connection between HP1β mobilisation and DNA repair. Although, as noted above, many questions remain to be answered concerning the events that trigger HP1 dispersal, and the mechanism by which it influences the repair of DNA lesions, our findings provide a starting point from which to further elucidate a hitherto unrecognized signaling cascade mediating the cellular response to DNA damage.

Our findings highlight the mounting evidence that structural alterations in chromatin are an intrinsic and essential feature of the mechanisms used by cells to sense and signal DNA lesions. Interestingly, we find in this instance that chromatin changes are brought about not by modifications in the histone code, but by phosphorylation of a protein, HP1, that translates the code. This illustrates the concept that transient chromatin alterations necessary for other important biological processes like transcription may also be triggered by changes in histone-code effector proteins, rather than the code itself.

Our findings implicate CK2 in the earliest cellular responses to DNA breakage, and underscore our limited understanding of how this nuclear kinase, although ubiquitously distributed, may nevertheless be activated (or act) focally. Given mounting evidence for CK2's role in several facets of the DNA damage response,[45,80-84] it seems likely that further CK2 substrates that participate in this pathway remain to be discovered. This should help in elucidating the role of this kinase in carcinogenesis,[91] and help to refine efforts to target it for cancer therapy.[91]

Our earlier work[45] highlights the role played by the rapid, transient mobilization of HP1 within seconds after DNA damage in paving the way for H2AX phosphorylation. We emphasize,

however, as supported by the data we show here, that this may represent only one facet of a complex picture. Clearly, HP1 re-accumulates over time on sites of damage from which it was mobilized, and this response, too, may contribute to the process of DNA damage sensing and repair, but in a functionally distinct way from the initial mobilization.

Finally, our work illustrates how advances in interventional microscopy and live-cell imaging techniques enable studies on dynamic and reversible biological events that occur within seconds after DNA damage. Indeed, such transient and subtle regulatory mechanisms based on protein turnover and binding affinity could also influence several other aspects of the DNA damage response, hitherto unrecognized by the conventional biochemical techniques thus far used to characterize them.

Acknowledgements

We acknowledge awards to A.R.V. from the Medical Research Council (N.A. and A.D.J.), and the Wellcome Trust (J.B.) and thank members of our laboratory for constructive criticism. A.D.J. was the recipient of a Gates Foundation studentship.

References

1. Flemming W. Zellsubstanz, Kern und Zelltheilung. Leipzig: F.C.W. Vogel 1882.
2. Heitz E. Das heterochromatin der moose. I Jahrb Wiss Botanik 1928; 69:762-818.
3. Passarge E. Emil Heitz and the concept of heterochromatin: longitudinal chromosome differentiation was recognized fifty years ago. Am J Hum Genet 1979; 31:4-7.
4. Craig JM. Heterochromatin—many flavours, common themes. Bioessays 2005; 27:17-28.
5. Grewal SI, Jia S. Heterochromatin revisited. Nat Rev Genet 2007; 8:35-46.
6. Dillon N. Heterochromatin structure and function. Biol Cell 2004; 96:631-7.
7. Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 2006; 441:349-53.
8. Strahl BD, Allis CD. The language of covalent histone modifications. Nature 2000; 403:41-5.
9. Downs JA, Nussenzweig MC, Nussenzweig A. Chromatin dynamics and the preservation of genetic information. Nature 2007; 447:951-8.
10. Kamakaka RT, Biggins S. Histone variants: deviants? Genes Dev 2005; 19:295-310.
11. Polo SE, Almouzni G. Chromatin assembly: a basic recipe with various flavours. Curr Opin Genet Dev 2006; 16:104-11.
12. Maison C, Almouzni G. HP1 and the dynamics of heterochromatin maintenance. Nat Rev Mol Cell Biol 2004; 5:296-304.
13. James TC, Elgin SC. Identification of a nonhistone chromosomal protein associated with heterochromatin in *Drosophila melanogaster* and its gene. Mol Cell Biol 1986; 6:3862-72.
14. Hake SB, Xiao A, Allis CD. Linking the epigenetic 'language' of covalent histone modifications to cancer. Br J Cancer 2004; 90:761-9.
15. Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, et al. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet 2005; 37:391-400.
16. Jones PA, Baylin SB. The epigenomics of cancer. Cell 2007; 128:683-92.
17. Corpet A, Almouzni G. Making copies of chromatin: the challenge of nucleosomal organization and epigenetic information. Trends Cell Biol 2008.
18. Bruno M, Flaus A, Stockdale C, Rencurel C, Ferreira H, Owen-Hughes T. Histone H2A/H2B dimer exchange by ATP-dependent chromatin remodeling activities. Mol Cell 2003; 12:1599-606.
19. Vicent GP, Nacht AS, Smith CL, Peterson CL, Dimitrov S, Beato M. DNA instructed displacement of histones H2A and H2B at an inducible promoter. Mol Cell 2004; 16:439-52.
20. Kouzarides T. Chromatin modifications and their function. Cell 2007; 128:693-705.
21. Jenuwein T, Allis CD. Translating the histone code. Science 2001; 293:1074-80.
22. Schotta G, Lachner M, Sarma K, Ebert A, Sengupta R, Reuter G, et al. A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. Genes Dev 2004; 18:1251-62.
23. Peters AH, O'Carroll D, Scherthan H, Mechtler K, Sauer S, Schofer C, et al. Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. Cell 2001; 107:323-37.
24. Rougeulle C, Chaumeil J, Sarma K, Allis CD, Reinberg D, Avner P, et al. Differential histone H3 Lys-9 and Lys-27 methylation profiles on the X chromosome. Mol Cell Biol 2004; 24:5475-84.
25. Bernstein BE, Kamal M, Lindblad-Toh K, Bekiranov S, Bailey DK, Huebert DJ, et al. Genomic maps and comparative analysis of histone modifications in human and mouse. Cell 2005; 120:169-81.
26. Roh TY, Cuddapah S, Zhao K. Active chromatin domains are defined by acetylation islands revealed by genome-wide mapping. Genes Dev 2005; 19:542-52.
27. Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC, et al. Active genes are tri-methylated at K4 of histone H3. Nature 2002; 419:407-11.
28. Dhalluin C, Carlson JE, Zeng L, He C, Aggarwal AK, Zhou MM. Structure and ligand of a histone acetyltransferase bromodomain. Nature 1999; 399:491-6.
29. Jacobson RH, Ladurner AG, King DS, Tjian R. Structure and function of a human TAFII250 double bromodomain module. Science 2000; 288:1422-5.
30. Owen DJ, Ornaghi P, Yang JC, Lowe N, Evans PR, Ballario P, et al. The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase gcn5p. EMBO J 2000; 19:6141-9.
31. Yang XJ. Lysine acetylation and the bromodomain: a new partnership for signaling. Bioessays 2004; 26:1076-87.
32. Cairns BR, Schlichter A, Erdjument-Bromage H, Tempst P, Kornberg RD, Winston F. Two functionally distinct forms of the RSC nucleosome-remodeling complex, containing essential AT hook, BAH and bromodomains. Mol Cell 1999; 4:715-23.
33. Kasten M, Szerlong H, Erdjument-Bromage H, Tempst P, Werner M, Cairns BR. Tandem bromodomains in the chromatin remodeler RSC recognize acetylated histone H3 Lys14. EMBO J 2004; 23:1348-59.
34. Soutourina J, Bordas-Le Floch V, Gendrel G, Flores A, Ducrot C, Dumay-Odelot H, et al. Rsc4 connects the chromatin remodeler RSC to RNA polymerases. Mol Cell Biol 2006; 26:4920-33.
35. Bannister AJ, Zegerman P, Partridge JF, Miska EA, Thomas JO, Allshire RC, et al. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 2001; 410:120-4.
36. Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 2001; 410:116-20.
37. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, et al. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 2002; 298:1039-43.
38. Czermin B, Melfi R, McCabe D, Seitz V, Imhof A, Pirrotta V. Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. Cell 2002; 111:185-96.
39. Kuzmichev A, Nishioka K, Erdjument-Bromage H, Tempst P, Reinberg D. Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. Genes Dev 2002; 16:2893-905.
40. Muller J, Hart CM, Francis NJ, Vargas ML, Sengupta A, Wild B, et al. Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. Cell 2002; 111:197-208.
41. Robinson PJ, An W, Routh A, Martino F, Chapman L, Roeder RG, et al. 30 nm chromatin fibre decompaction requires both H4-K16 acetylation and linker histone eviction. J Mol Biol 2008; 381:816-25.
42. Dorigo B, Schalch T, Bystricky K, Richmond TJ. Chromatin fiber folding: requirement for the histone H4 N-terminal tail. J Mol Biol 2003; 327:85-96.
43. Shogren-Knaak M, Ishii H, Sun JM, Pazin MJ, Davie JR, Peterson CL. Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science 2006; 311:844-7.
44. Lomberk G, Bensi D, Fernandez-Zapico ME, Urrutia R. Evidence for the existence of an HP1-mediated subcode within the histone code. Nat Cell Biol 2006; 8:407-15.
45. Ayoub N, Jeyasekharan AD, Bernal JA, Venkitaraman AR. HP1-beta mobilization promotes chromatin changes that initiate the DNA damage response. Nature 2008; 453:682-6.
46. Kruhlak MJ, Celeste A, Dellaire G, Fernandez-Capetillo O, Muller WG, McNally JG, et al. Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. J Cell Biol 2006; 172:823-34.
47. Rubbi CP, Milner J. p53 is a chromatin accessibility factor for nucleotide excision repair of DNA damage. EMBO J 2003; 22:975-86.
48. Ziv Y, Bielopolski D, Galanty Y, Lukas C, Taya Y, Schultz DC, et al. Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. Nat Cell Biol 2006; 8:870-6.
49. Goodarzi AA, Noon AT, Deckbar D, Ziv Y, Shiloh Y, Lobrich M, et al. ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. Mol Cell 2008; 31:167-77.
50. Fernandez-Capetillo O, Lee A, Nussenzweig M, Nussenzweig A. H2AX: the histone guardian of the genome. DNA Repair (Amst) 2004; 3:959-67.
51. Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 1998; 273:5858-68.
52. Fillingham J, Keogh MC, Krogan NJ. GammaH2AX and its role in DNA double-strand break repair. Biochem Cell Biol 2006; 84:568-77.
53. Tamburini BA, Tyler JK. Localized histone acetylation and deacetylation triggered by the homologous recombination pathway of double-strand DNA repair. Mol Cell Biol 2005; 25:4903-13.

54. Costelloe T, Fitzgerald J, Murphy NJ, Flaus A, Lowndes NF. Chromatin modulation and the DNA damage response. Exp Cell Res 2006; 312:2677-86.
55. Paull TT, Rogakou EP, Yamazaki V, Kirchgessner CU, Gellert M, Bonner WM. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. Curr Biol 2000; 10:886-95.
56. Celeste A, Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova OA, et al. Genomic instability in mice lacking histone H2AX. Science 2002; 296:922-7.
57. Celeste A, Difilippantonio S, Difilippantonio MJ, Fernandez-Capetillo O, Pilch DR, Sedelnikova OA, et al. H2AX haploinsufficiency modifies genomic stability and tumor susceptibility. Cell 2003; 114:371-83.
58. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature 2003; 421:499-506.
59. Park JH, Park EJ, Lee HS, Kim SJ, Hur SK, Imbalzano AN, et al. Mammalian SWI/SNF complexes facilitate DNA double-strand break repair by promoting gamma-H2AX induction. EMBO J 2006; 25:3986-97.
60. Soutoglou E, Misteli T. Activation of the cellular DNA damage response in the absence of DNA lesions. Science 2008; 320:1507-10.
61. Ikura T, Tashiro S, Kakino A, Shima H, Jacob N, Amunugama R, et al. DNA damage-dependent acetylation and ubiquitination of H2AX enhances chromatin dynamics. Mol Cell Biol 2007; 27:7028-40.
62. Kusch T, Florens L, Macdonald WH, Swanson SK, Glaser RL, Yates JR, 3rd, et al. Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. Science 2004; 306:2084-7.
63. Eissenberg JC, Morris GD, Reuter G, Hartnett T. The heterochromatin-associated protein HP-1 is an essential protein in Drosophila with dosage-dependent effects on position-effect variegation. Genetics 1992; 131:345-52.
64. James TC, Eissenberg JC, Craig C, Dietrich V, Hobson A, Elgin SC. Distribution patterns of HP1, a heterochromatin-associated nonhistone chromosomal protein of Drosophila. Eur J Cell Biol 1989; 50:170-80.
65. Eissenberg JC, Elgin SC. The HP1 protein family: getting a grip on chromatin. Curr Opin Genet Dev 2000; 10:204-10.
66. Lechner MS, Begg GE, Speicher DW, Rauscher FJ, 3rd. Molecular determinants for targeting heterochromatin protein 1-mediated gene silencing: direct chromoshadow domain-KAP-1 corepressor interaction is essential. Mol Cell Biol 2000; 20:6449-65.
67. Fuks F, Hurd PJ, Deplus R, Kouzarides T. The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. Nucleic Acids Res 2003; 31:2305-12.
68. Aagaard L, Laible G, Selenko P, Schmid M, Dorn R, Schotta G, et al. Functional mammalian homologues of the Drosophila PEV-modifier Su(var)3-9 encode centromere-associated proteins which complex with the heterochromatin component M31. EMBO J 1999; 18:1923-38.
69. Ayoub N, Noma K, Isaac S, Kahan T, Grewal SI, Cohen A. A novel jmjC domain protein modulates heterochromatization in fission yeast. Mol Cell Biol 2003; 23:4356-70.
70. Zofall M, Grewal SI. Swi6/HP1 recruits a JmjC domain protein to facilitate transcription of heterochromatic repeats. Mol Cell 2006; 22:681-92.
71. Cheutin T, McNairn AJ, Jenuwein T, Gilbert DM, Singh PB, Misteli T. Maintenance of stable heterochromatin domains by dynamic HP1 binding. Science 2003; 299:721-5.
72. Festenstein R, Pagakis SN, Hiragami K, Lyon D, Verreault A, Sekkali B, et al. Modulation of heterochromatin protein 1 dynamics in primary Mammalian cells. Science 2003; 299:719-21.
73. Schmiedeberg L, Weisshart K, Diekmann S, Meyer Zu Hoerste G, Hemmerich P. High- and low-mobility populations of HP1 in heterochromatin of mammalian cells. Mol Biol Cell 2004; 15:2819-33.
74. Jung-Ae Kim MK, Farokh Dotiwala, André Nussenzweig and James E. Haber. Heterochromatin is refractory to γH2AX modification in yeast and mammals, The Journal of Cell Biology 2007; 178:209-18.
75. Cowell IG, Sunter NJ, Singh PB, Austin CA, Durkacz BW, Tilby MJ. gammaH2AX foci form preferentially in euchromatin after ionising-radiation. PLoS ONE 2007; 2:1057.
76. Fischle W, Tseng BS, Dormann HL, Ueberheide BM, Garcia BA, Shabanowitz J, et al. Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. Nature 2005; 438:1116-22.
77. Solovjeva LV, Svetlova MP, Chagin VO, Tomilin NV. Inhibition of transcription at radiation-induced nuclear foci of phosphorylated histone H2AX in mammalian cells. Chromosome Res 2007; 15:787-97.
78. Pinna LA. Protein kinase CK2: a challenge to canons. J Cell Sci 2002; 115:3873-8.
79. Meggio F, Pinna LA. One-thousand-and-one substrates of protein kinase CK2? Faseb J 2003; 17:349-68.
80. Loizou JI, El-Khamisy SF, Zlatanou A, Moore DJ, Chan DW, Qin J, et al. The protein kinase CK2 facilitates repair of chromosomal DNA single-strand breaks. Cell 2004; 117:17-28.
81. 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.
82. Melander F, Bekker-Jensen S, Falck J, Bartek J, Mailand N, Lukas J. Phosphorylation of SDT repeats in the MDC1 N terminus triggers retention of NBS1 at the DNA damage-modified chromatin. J Cell Biol 2008; 181:213-26.
83. Spycher C, Miller ES, Townsend K, Pavic L, Morrice NA, Janscak P, et al. Constitutive phosphorylation of MDC1 physically links the MRE11-RAD50-NBS1 complex to damaged chromatin. J Cell Biol 2008; 181:227-40.
84. Wu L, Luo K, Lou Z, Chen J. MDC1 regulates intra-S-phase checkpoint by targeting NBS1 to DNA double-strand breaks. Proc Natl Acad Sci USA 2008; 105:11200-5.
85. Cheung WL, Turner FB, Krishnamoorthy T, Wolner B, Ahn SH, Foley M, et al. Phosphorylation of histone H4 serine 1 during DNA damage requires casein kinase II in *S. cerevisiae*. Curr Biol 2005; 15:656-60.
86. Shimada A, Dohke K, Sadaie M, Shinmyozu K, Nakayama J, Urano T, et al. Phosphorylation of Swi6/HP1 regulates transcriptional gene silencing at heterochromatin. Genes Dev 2009; 23:18-23.
87. Eissenberg JC, Ge YW, Hartnett T. Increased phosphorylation of HP1, a heterochromatin-associated protein of Drosophila, is correlated with heterochromatin assembly. J Biol Chem 1994; 269:21315-21.
88. Zhao T, Eissenberg JC. Phosphorylation of heterochromatin protein 1 by casein kinase II is required for efficient heterochromatin binding in Drosophila. J Biol Chem 1999; 274:15095-100.
89. Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER, 3rd, Hurov KE, Luo J, et al. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science 2007; 316:1160-6.
90. Sharma GG, Hwang KK, Pandita RK, Gupta A, Dhar S, Parenteau J, et al. Human heterochromatin protein 1 isoforms HP1(Hsalpha) and HP1(Hsbeta) interfere with hTERT-telomere interactions and correlate with changes in cell growth and response to ionizing radiation. Mol Cell Biol 2003; 23:8363-76.
91. Sarno S, Pinna LA. Protein kinase CK2 as a druggable target. Mol Biosyst 2008; 4:889-94.