

Choreographing the DNA damage response

PP6 joins the dance

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Key words: ionizing radiation, DNA double strand breaks, protein phosphatase, histone H2AX, DNA-dependent protein kinase

DNA damaging agents such as ionizing radiation (IR), as well as naturally occurring processes such as V(D)J recombination, lead to the formation of DNA double strand breaks (DSBs), which, if not repaired faithfully and efficiently can lead to genomic instability, a hallmark of cancer.¹ Induction of DSBs leads to activation of cell cycle checkpoints, which result in a temporary halt in cell cycle progression, allowing more time for damage to be repaired prior to entry into or progression of S phase or entry into mitosis, as well as activation of the repair pathways non-homologous end-joining (NHEJ) and homologous recombination repair.¹ If the damage is too severe or cannot be repaired, cell death or extended growth arrest ensues.² Together, these repair and signaling pathways are referred to as the DNA damage response (DDR).¹

One of the key events in the DDR is DNA damage-induced phosphorylation of histone variant H2AX on serine 139 (in human cells), to a form that is commonly referred to as γ -H2AX. IR-induced phosphorylation of H2AX is carried out by the phosphatidylinositol-3 kinase-like (PIKK) protein kinases, Ataxia-Telangiectasia Mutated (ATM) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and occurs over megabases of chromatin either side of the DSB. γ -H2AX interacts directly with Mediator of DNA damage Checkpoint 1 (MDC1), which leads to recruitment of multiple proteins including the E3 ubiquitin ligases, RNF8 and RNF168, and 53BP1 to the break site.³ Accumulation of these proteins at sites of DNA damage, as well as their associated post-translational modifications such as

phosphorylation and ubiquitination, can be visualized by microscopy, and these protein assemblies are referred to as IR-induced foci (IRIF). Although the precise function of IRIF is still unclear, they are likely involved in directing cell cycle arrest, as well as marking sites of DNA damage in heterochromatic regions that are difficult to repair.²

H2AX phosphorylation and foci formation is a highly dynamic process. γ -H2AX is formed within minutes after IR and, in repair-competent cells, γ -H2AX generally persists for several hours before returning to background levels. However, in cells defective in key components of NHEJ, foci may persist for 24 hours or longer, consistent with the presence of unrepaired DSBs. Thus, γ -H2AX is widely regarded as an indication of the DSB repair capacity of the cell.² However, since foci also represent sites of phosphorylation, loss of IRIF also reflects γ -H2AX dephosphorylation. Indeed, two members of the phosphoprotein phosphatase 2A (PP2A)-like family, PP2A and PP4, have been shown to dephosphorylate γ -H2AX in vitro and to regulate γ -H2AX foci stability in vivo.^{4,7} Recently, the third member of this protein family, PP6, was also shown to regulate H2AX phosphorylation and foci stability in response to IR.⁸ Moreover, both PP2A^{8,9} and PP6,^{8,10} interact with DNA-PK which is recruited to IR-induced DSBs in the initial stages of NHEJ.¹¹ Furthermore, downregulation of PP2A inhibits DSB repair,⁹ and, like PP4, PP6 is required for release from the G₂/M checkpoint after DNA damage.^{7,8} Together, these studies suggest that DNA-PK recruits PP2A and PP6 to sites of DNA damage, where they dephosphorylate γ -H2AX and possibly other

proteins present at IRIF, thus regulating IRIF disassembly (Fig. 1).

These new findings raise several interesting questions. For example, why are all three members of the PP2A-like phosphatase family involved in dephosphorylation of γ -H2AX and, consequently, disassembly of IRIF? One possibility is that different PP2A-family members are involved in dephosphorylating different populations of γ -H2AX, such as those formed in heterochromatic regions versus those in euchromatin. Alternatively, different protein phosphatases may be required to dephosphorylate γ -H2AX located at different distances from the DSB or for γ -H2AX that has been released from chromatin by the action of chromatin remodeling complexes and is dephosphorylated in the nucleoplasm. In addition, different protein phosphatases may dephosphorylate γ -H2AX in response to different forms of DNA damage. Indeed, PP4 is required for dephosphorylation of replication-induced γ -H2AX.⁷ It is also interesting to note that, in addition to PP6 and PP2A, DNA-PKcs has also been reported to interact with PP5 and PP1 (reviewed in ref. 8) suggesting that it may act as a targeting subunit for multiple protein phosphatases. It will be interesting to determine whether these various protein phosphatases target other protein substrates present at IRIF, and, if so, how dephosphorylation of these proteins regulates the kinetics of foci assembly and disassembly as well as downstream events, such as repair and cell cycle checkpoint arrest. Finally, these studies highlight the importance of protein dephosphorylation in regulating the DDR, reminding us that turning off the

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Submitted: 01/27/10; Accepted: 01/27/10

Previously published online: www.landesbioscience.com/journals/cc/article/11321

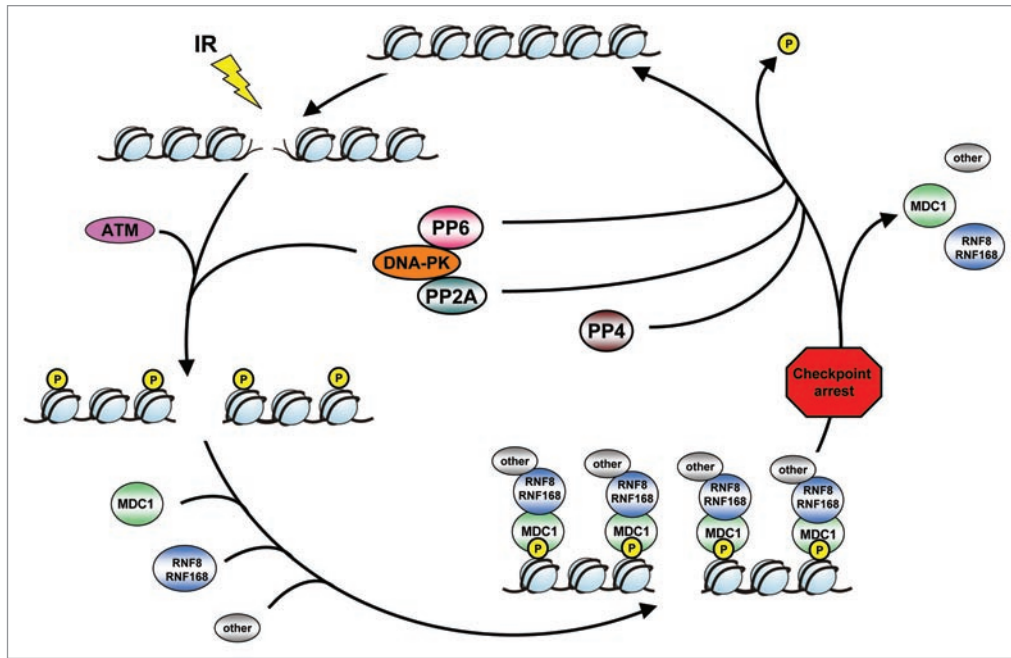


Figure 1. Model for the role of DNA-PK and PP2A-like protein phosphatases in the dephosphorylation of γ -H2AX. IR results in the formation of DSBs, which result in activation of DNA-PK and ATM and phosphorylation of histone H2AX on serine 139 to form γ -H2AX (indicated by yellow circles with P). MDC1 binds directly to γ -H2AX, providing a scaffold for recruitment of RNF8, RNF168 and other proteins to IRIF. DNA-PK interacts with protein phosphatases PP2A and PP6 and is required for repair of DSBs by NHEJ, providing a potential mechanism for recruitment of γ -H2AX phosphatases to the break. In subsequent steps, MDC1, RNF8, RNF168 and other proteins dissociate from IRIF and γ -H2AX is dephosphorylated, signaling repair of the DSB.

cellular signal may be as just as important and as intricately regulated as turning it on. Moreover, the molecules involved in attenuating the DDR may be just as important targets for therapeutic drug development as those involved in its activation.

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