DNA Repair 32 (2015) 58–65

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Emerging role of protein phosphatases changes the landscape of phospho-signaling in DNA damage response

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ARTICLE INFO

Article history:
Available online 1 May 2015

Keywords:
Protein phosphatase
Dephosphorylation
ATM
KAP1
CTIP
EXO1
53BP1
XRCC4
YEN1
BLM

ABSTRACT

Phosphorylation signaling networks have primarily been studied from an activation perspective, with protein phosphatases viewed as simple counter-balances that functioned passively in the wake of kinase activity. Indeed, there have been only sporadic efforts to investigate the independent role of phosphatases in DNA damage response (DDR). However, global phosphoproteomic analysis of the DDR revealed that over one-third of observed phosphorylation sites were down-regulated within minutes of DNA damage, suggesting a more robust role for phosphatases in DNA repair. Consistent with these observations, recent studies reveal that dephosphorylation of DNA repair factors during specific phases of the cell cycle may be a pre-requisite for their participation in the DDR. Here, we summarize recent literature and speculate on the emerging role of phosphatases in the DDR.

1. Dephosphorylation during DNA damage response

The traditional view of DNA damage induced phospho-signaling entails a single burst of phosphorylation of largely serine (Ser)/(Thr) residues that is responsible for the activation of DNA repair factors and checkpoint proteins. In the course of repair, the phosphorylation gradually dissipates, allowing cell cycle to resume [1,2]. Recent studies investigating the dynamics of phosphorylation following DNA damage show that there is considerable variation in the kinetics and direction of phosphoproteomic changes. 753 phosphorylation sites mapping to 394 proteins were altered in response to a radiomimetic agent, neocarzinostatin (NCS), and an astounding 342 of these sites represented dephosphorylation events [2]. Over one-third of the captured phosphopeptides were dephosphorylated within minutes of DNA damage. These data suggest that protein phosphatases not only play a role in counter-acting DSB-induced phosphorylation events, but also play a primary role in initiating the repair process. The human genome encodes 514 protein kinases, but only 147 protein phosphatases [3]. It is therefore assumed that each phosphatase has a large number of substrates, potentially influencing multiple signaling pathways. Also the relatively modest number of phosphatase subunits as compared to kinases suggests that the former may rely on a wider repertoire of molecular mechanisms to coordinate regulation. Hence, a major challenge in the study of phosphatases has been to experimentally capture the diverse molecular architecture that mediates

Abbreviations: 53BP1, p53 binding protein 1; ATM, ataxia-telangiectasia mutated; ATR, ataxia telangiectasia and Rad3 related; BLM, blooms syndrome RecQ helicase-like, human homolog of SGS1; BRCA1, breast cancer 1, early onset; CDC, cell division cycle; CDK, cyclin dependent kinase; CHD3, chromodomain-helicase-DNA-binding protein; CO, crossover product from recombination intermediate resolution; CTIP, C-terminal binding protein-interacting protein; DDR, DNA damage response; DSB, DNA double-stranded breaks; DUSP, dual-specificity phosphatases; EXO1, exonuclease 1; FEAR, (Cdc) fourteen early-anaphase release; FHA, forkhead-associated domain; GEN1, GEN1Holliday junction 5' flap endonuclease; HJ, Holliday junction; HU, hydroxyurea; HR, homologous recombination; KAP1, Kruppel-associated box protein 1; LIF1, ligase-interacting factor 1; MDC1, mediator of DNA-damage checkpoint 1; MEN, mitotic exit network; MMS4, human crossover junction endonuclease EME1; MUS81, human crossover junction endonuclease MUS81; MRN, MRE11-RAD50-NBS1; NES, nuclear exclusion signal; NHEJ, non-homologous end joining; NCO, noncrossover product from recombination intermediate resolution; NCS, neocarzinostatin; PIP, PP1-interacting protein; PLK, Polo-like kinase; PML, promyelocytic leukemia protein; PNK, polynucleotide kinase; PPM, Mg2+/Mn2+-dependent phosphatases; PPM1D, Mg2+/Mn2+-dependent phosphatases 1D, Wip1; PPP, protein phosphatases; PTIP, PAX interacting (with transcription-activation domain) protein1; RPA, replication protein A; RAD51, RAD51 recombinase; RIF1, replication timing regulatory factor 1; SCE, sister chromatid exchange; Ser, serine; Thr, threonine; WIP1, wild-type p53-induced phosphatase; XLF1, XRCC4-like factor 1; XRCC4, X-ray repair complementing defective repair in Chinese hamster cells 4; YEN1, Yen1Holliday junction resolvase.

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http://dx.doi.org/10.1016/j.dnarep.2015.04.014
1568-7864/

Table 1
Ser/Thr phosphatases involved in DSB repair and checkpoint control.

Substrate	Phosphatase	Effect on the substrate
Sensors and transducers		
H2AX	PP1/PP2A/PP4/PP6/WIP1	Attenuation
ATM	PP1/WIP1	Attenuation
ATM	PP5	Activation
ATR	PP5	Activation
PARP	PP5	Activation
BRCA1	PP1	Activation
53BP1	PP5	Attenuation
KAP1	PP1	Attenuation
Checkpoint		
P53	PP1/PP2A/WIP1	Attenuation
MDM2	WIP1	Attenuation
CHEK1	PP2A/WIP1	Attenuation
CHEK2	PP2A	Attenuation
NHEJ		
DNA-PKcs	PP2A/PP5/PP6	Activation
Ku70/80a	PP2A	Activation
HR		
RPA2	PP2A/PP4	Attenuation

phosphatase activity and the targets of these phosphatase complexes. A number of well-established DNA repair factors targeted by phosphatases is listed in Table 1 and summarized in our previous review [4]. In this perspective article we focus on recent studies that demonstrate or implicate dephosphorylation as a critical regulatory event throughout DDR. The cases we highlight here demonstrate that protein phosphatases function to 1) reset activated DDR factors to the initial homeostatic state following the repair of damaged DNA, thereby priming these factors for their role in the next round of the DNA damage response; and 2) remove constitutive phosphorylation that inhibits the function DNA repair factors, effectively activating these proteins.

2. Protein phosphatases in DDR

Ser/Thr phosphatases are classified based on sequence, structure, and biochemical properties, including metal co-factor dependence and sensitivity to phosphatase inhibitors. The main classes of protein phosphatases (PPP) that have been implicated in DDR are PP1, PP2A-like phosphatases, and Mg^{2+}/Mn^{2+}-dependent phosphatases (PPM) [4].

PP1 is an ubiquitously expressed phosphatase involved in cellular functions ranging from RNA processing, mitotic progression, to checkpoint activation and DNA repair [4]. PP1 has three catalytic subunits— PP1α, PP1β and PP1γ [3]. These catalytic subunits form at least 650 distinct complexes with PP1-interacting proteins (PIPs) [4]. These PIPs include substrates, substrate-targeting regulatory subunits, and substrate regulators [5,6]. Most PP1 targets identified thus far harbor an RVxF motif which is thought to be the recognition motif for PP1 [3].

The PP2A-like phosphatases family includes PP2AC, PP4C and PP6C [4]. Protein phosphatases in this family share significant amino acid sequence homology and similar sensitivity to chemical inhibitors, such as okadaic acid. The phosphatase catalytic subunit forms multimeric complex with regulatory and scaffolding subunits to recognize specific substrates. PP2A is one of the most well studied phosphatases and is involved in many cellular functions including cell metabolism and survival [7]. PP2A typically functions as a heterotrimeric complex, in which the catalytic subunit (C) binds to a scaffold subunit (A) at its C-terminus, and a substrate targeting subunit (B) proximal to its active site. In humans there are two PP2A catalytic subunits (PP2ACα and PP2ACβ), two scaffold subunits (PR65α and PR65β) and a myriad of substrate-targeting regulatory subunits classified into subfamilies of PR55, PR61, and PR72 [3]. PP4C shares 65% sequence homology with PP2AC and associates with regulatory subunits PP4R1, PP4R2, PP4R3α, PP4R3β, and PP4R4 [4]. Human PP6C was identified by its rescue of temperature-sensitive mutants of Sit4, which is homolog of PP6C in *Saccharomyces cerevisiae* [8]. PP6 functions as a heterotrimeric complex containing PP6C, one of the three regulatory subunits PP6R1, R2, or R3, and proteins with ankyrin repeat domains [4].

PP2C is part of the Mg^{2+}/Mn^{2+}-dependent PPM, and a well-studied member of this family is wild-type p53-induced phosphatase (WIP1, also known as PP2C or PPM1D), named so due to its induced expression is p53-dependent after DNA damage [9]. Concordant with its function in deactivating tumor suppressors, WIP1 is an oncogene amplified and overexpressed in many human cancers [10].

CDC14 belongs to the family of highly conserved dual-specificity phosphatases (DUSPs). DUSPs are characterized by their ability to dephosphorylate phosphotyrosine, phoshoserine and phoshothreonine residues in their substrates [11]. CDC14 of *S. cerevisiae* is regulated by CDC14 early-anaphase release (FEAR) pathway and mitotic exit network (MEN) in terms of its subcellular localization. FEAR and MEN pathways signal the release of CDC14 from nucleolus into nucleoplasm and cytoplasm [12]. CDC14 function during the transition of metaphase to anaphase for anaphase spindle stabilization and mitotic exit, via reversal of cyclin-depedent kinase 1 (CDK1)-mediated phosphorylation [12].

3. Dephosphorylation regulates DSB repair in a cell cycle-dependent manner

DNA double-strand breaks (DSBs) are the most deleterious lesion for the cells—a single unrepaired DSB may be sufficient for inducing apoptosis [13]. Two major mechanistically distinct pathways, homologous recombination (HR) and non-homologous end joining (NHEJ), have evolved to deal with DSBs and are regulated by highly conserved factors [14]. HR requires an undamaged homologous DNA template to restore information lost in the damaged DNA strand with high fidelity, while error-prone NHEJ rapidly processes and joins broken DNA ends [14]. The relative contribution of these pathways varies in cell types and phases of the cell cycle—typically NHEJ is favored in the pre-replicative (G0/G1) phase, while HR dominates in the replicative (S) phase [14]. The spatial and temporal recruitment of DSB repair factors to the site of the DNA break is tightly regulated to ensure efficient and appropriate execution of these pathways. Post-translational modifications, including phosphodynamics, of these proteins are central to this regulatory step.

3.1. ATM

The current widely accepted model of activation of ataxia-telangiectasia mutated (ATM) entails rapid auto-phopshorylation at Ser1981 in response to DNA damage [15]. This phosphorylation event induces the dissociation of ATM dimer distributed in the nucleus, allowing the release of active monomeric ATM to localize to damaged chromatin and phosphorylate downstream checkpoint proteins [16]. The protein phosphatase PP2A has been previously implicated in regulating ATM activity in undamaged cells [17], but the molecular details of this regulation were not clear. PP2AC-B55α holoenzyme interacts directly with ATM and catalyzes dephosphorylation of ATM at Ser367, Ser1893 and Ser1981 *in vitro* [18]. The PP2AC-B55α complex regulates ATM activity in a DNA damage- and time- dependent manner. The impact of PP2AC–B55α complex on ATM diminishes 15 min post-irradiation and is restored again