Protein post-translational modifications and the DNA damage response

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Abstract
Gene expression can be regulated in many ways at the level of transcription or translation. The quickest way is to modify the gene product or protein. Post-translational modifications (PTMs) of proteins can alter their activity, interaction partners, complex affiliation, subcellular localization, or stability in response to changes in the cellular environment. There is a well-established and diverse range of PTMs. Phosphorylation is best characterized due to its essential role in a wide range of cellular processes such as the DNA damage response (DDR).
Keywords: posttranslational modifications, DNA damage response, DNA damage checkpoint, phosphorylation.

Introduction

Regulation of gene expression can occur at many levels, and one of the most commonly studied has been at the level of protein post-translational control. Post-translational modification of a protein is a biochemical mechanism in which amino-acid residues in a protein are covalently modified. These modifications not only confer the diversity of protein functions, but also regulate protein folding, protein-protein interactions, protein stability, and allosteric regulation of enzyme activity. In addition, they can alter interactions with other macromolecules or cellular localization, and play an important role in controlling protein homeostasis and quality control. Unlike slower transcriptional or translational responses, modification of existing proteins allows dynamic, and often reversible, regulation of cellular physiology in response to changing environments. To date, more than 200 different types of PTMs, such as the addition of small chemical groups, small peptides, carbohydrates, or lipids, have been detected on proteins (Jensen 2006). This diversity of PTMs contributes to different outcomes for the attached protein and the cellular processes that it is involved in (Prabakaran et al. 2012).

In this review, we will introduce PTMs that are important for the cellular response to DNA damage. Among these PTMs, protein phosphorylation plays essential roles in nearly every aspect of cell life, regulating many signaling pathways and a remarkably wide number of cellular processes such as cell-cycle progression and the DDR. Subsequently, we will briefly focus specifically on how the DNA damage checkpoint, orchestrated by kinases described below, utilizes phosphorylation events to exemplify the rapid response to DNA damage mediated by a PTM.

Common types of PTMs in the response to DNA damage

The cellular response to DNA damage requires rapid and reversible PTMs to quickly respond to genomic perturbations. These consist mostly of phosphorylation, ubiquitination, sumoylation, acetylation, and methylation (Polo and Jackson 2011). These modifications are highly dynamic and exist in the presence of opposing enzymatic activities between the catalyst that adds and

the catalyst that removes the modification. PTMs are an essential part of many signal transduction pathways, where a signal is sensed and transmitted through a signaling cascade. These signaling cascades often utilize enzymes that add, remove, and read the modification. Our knowledge of these PTMs may be reflected by their relative abundance in the cell (Table 1), but all serve to alter the function or stability of the protein that it modifies. Furthermore, PTMs are not mutually exclusive. For example, phosphorylation is often a primer for subsequent ubiquitination or sumoylation of a substrate (Dantuma and van Attikum 2016).

Modification	Residues Modified	% of proteome	Functional consequences
Phosphorylation	S, T, Y	~ 30%	Modifies protein-protein interactions, localization, enzymatic activity
Ubiquitination	L	~ 20%	Modifies stability, protein-protein interactions
Sumoylation	L	~ 8%	Modifies protein-protein interactions, localization
Acetylation	L	~ 10%	Modifies DNA binding properties, stability, protein-protein interactions
Methylation	L, R	> 1%	Modifies protein-protein interactions, stability, localization, and DNA binding

Table 1. Common types of PTMs (% estimated from mass spectrometry screens; see text for references). Single letter amino acid code corresponds to serine (S), threonine (T), tyrosine (Y), lysine (L), and arginine (R). For more thorough reviews on each PTM, the authors refer the reader to recent articles: phosphorylation (Johnson 2009), ubiquitination (Swatek and Komander 2016), sumoylation (Eifler and Vertegaal 2015), acetylation (Verdin and Ott 2015), and methylation (Biggar and Li 2015).

Protein phosphorylation is the most abundant (Table 1) and the best characterized PTM in eukaryotes due to its essential role in a wide range of cellular processes. Phosphorylation is an essential part of many signal transduction pathways that contain protein kinases, phosphatases, and phosphoprotein sensing proteins (Cohen 2002; Hunter 2014). This reversible modification involves the addition of a phosphate group from ATP to serine, threonine or tyrosine residues by protein kinases while protein phosphatases are able to remove the phosphate group and reverse the phosphorylation effect on the target protein (Lim and Pawson 2010; Beltrao et al. 2013; Kyriakis 2014). It is estimated that a third of mammalian proteins are phosphorylated at any given time (Mann et al. 2002), implying its extensive use in the cell. Protein kinases are typically more specific than phosphatases, which usually exhibit lower specificity for their targets. All principal players of the DDR are kinases and as such, they modify their direct substrates by phosphorylation. In addition, the DDR kinases require auto-phosphorylation, and thus, phosphorylation is essential for the activation and orchestration of the checkpoint. Protein dephosphorylation may be a regulatory event as important as phosphorylation, but it has not been extensively documented in the DDR (Bensimon et al. 2010).

Ubiquitin is a small 76 amino acid peptide that can be covalently attached to lysine residues of

substrates via an enzymatic cascade. Ubiquitination is also an abundant form of PTM and over 4200 ubiquitinated proteins were identified in the human proteome (Wagner et al. 2011). It is best known for its role in marking proteins, in the form of a polyubiquitin chain, for degradation by the proteasome. However, the attachment of a single ubiquitin (mono) onto a protein instead usually alters its localization or complex affiliation (Hicke 2001). Intriguingly, ubiquitin itself can be ubiquitinated on any of its seven lysine residues and therefore can achieve an additional layer of regulation based on the type of chains that are formed (Kim et al. 2007). However, the biological implication of atypical ubiquitin chains is not yet known. It has been predicted that there are about 600 ubiquitin ligases in the human genome (Li et al. 2008), but the substrates of the majority of these ligases remain a mystery. Conversely, there are close to 100 deubiquitinating enzymes or proteases that remove ubiquitin from proteins (Hutchins et al. 2013). Ubiquitination is another major component of the cellular response to DNA damage (Brinkmann et al. 2015).

SUMO is a small ubiquitin-like modifier and similar to ubiquitin, SUMO can be attached to lysine residues of a substrate via an analogous, but distinct, enzymatic cascade. A screen in 2014 revealed over 1600 sumoylated proteins in the proteome (Hendriks et al. 2014). Many DNA repair proteins are sumolyated around sites of DNA damage and this has been shown to be important for the proper interaction of repair proteins (Cremona et al. 2012; Hendriks et al. 2015).

Lysine acetyltransferases catalyze the transfer of an acetyl group from acetyl-CoA to the terminal amine group of the side chain of a lysine residue on a protein, altering protein structure and interactions. In contrast, deacetylases catalyze the reverse reaction. Acetylation were originally identified specifically on histones and was thought to be specific to histones, but a number of studies in recent years have shown that many non-histone proteins are acetylated and these proteins are involved in a number of processes, including DNA damage repair, and establish acetylation as an important global PTM (Choudhary et al. 2009; Zhao et al. 2010).

Methyltransferases catalyze the transfer of a methyl group onto the lysine and arginine residues of a protein in an S-adenosyl methionine dependent manner. Methylation eliminates the negative charge on the protein and increases the hydrophobic and basic property of the lysine residue. Although it is important in the response to DNA damage (Auclair and Richard 2013; Biggar and Li 2015), methylation occurs the least frequently in the cell with just about 140 proteins identified in screens (Bremang et al. 2013).

The DNA damage response

The faithful transfer of information from one generation to another requires the maintenance of genome integrity. In addition to high accuracy in DNA replication, the cell must also survive spontaneous or induced DNA damage while minimizing mutations. To achieve this goal, a highly conserved signal transduction pathway exists to monitor damages to DNA. The DDR initiates via sensor kinases that quickly detect alterations in DNA, mediator kinases that amplify the signal, and effector kinases that mount a global cellular response to the genomic insult (Melo and Toczyski 2002; Ciccia and Elledge 2010). Mutations in components of the DDR machinery as well as mutations in targets of the DDR are associated with neurological

disorders, genome stability and predisposition to cancer (Bartek and Lukas 2003).

In mammals, the central players of the checkpoint response are the kinase signaling proteins ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3 related) belonging to the superfamily of phosphatidylinositol-3-kinase-related kinases (Abraham 2001). ATM and ATR kinases are the prototype transducers that are rapidly activated after DNA damage and replication stress to initiate the DNA damage checkpoint-signaling pathway leading to cell cycle delay, DNA repair, and apoptosis (Figure 1). They have overlapping functions and similar substrate specificity *in vivo,* but are not redundant (Keegan et al. 1996; Zhao and Piwnica-Worms 2001; O'Neill et al. 2002). Activated ATM and ATR phosphorylate serine or threonine residues that are followed by glutamine (SQ/TQ motifs) on a large number of downstream substrates near the site of the damage (Shiloh 2003; Abraham 2004). These include phosphorylation of p53 (tumor suppressor), the histone variant H2AX, which is thought to help recruit other factors, such as 53BP1 (p53 binding protein), BRCA1 (breast cancer 1), and MDC1 (mediator of DNA damage checkpoint 1) (Burma et al. 2001; Bensimon et al. 2011). Among the key substrates are the serine–threonine checkpoint effector kinases, CHK1 (Checkpoint kinase 1) and CHK2 (Checkpoint kinase 2), which are selectively phosphorylated and activated by ATR and ATM respectively to trigger a wide range of distinct downstream responses (Jazayeri et al. 2006; Cai et al. 2009; Smith et al. 2010). CHK1 and CHK2 diffuse across the nucleoplasm and phosphorylate their substrates, including p53 and CDC25 (phosphatase cell division cycle 25), to regulate cell cycle progression (Bartek and Lukas 2003). Mutations that disrupt the phosphorylation of ATM, ATR, CHK1, and CHK2 fail to activate the checkpoint, indicating the significance of phosphorylation in the DDR signaling cascade (Capasso et al. 2002; Xu et al. 2002; Kozlov et al. 2011; Liu et al. 2011).

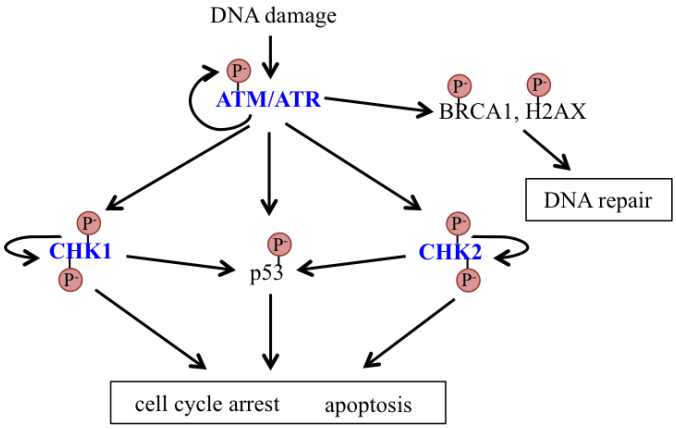

Figure 1. Events of phosphorylation coordinate the DDR. Activated ATR and ATM promote the phosphorylation of CHK1 and CHK2 respectively, and p53. Upon exposure to a genotoxic agent, the repair of a large amount of damage requires cell cycle arrest, or apoptosis if many attempts to repair DNA damage fail. ATM/ATR also triggers H2AX and BRCA1 phosphorylation to facilitate recruitment of DDR factors to repair the damage.

Concluding remarks

Mass spectrometry has allowed easy and rapid identification of PTMs and has lead to proteomic screens to identify specific PTMs, the residues involved, and proteins associated with a particular PTM. However, validation is lagging behind discovery and our knowledge of the biological significance of these PTMs is limited. This is especially true in the many attempts to identify targets of the DDR (Matsuoka et al. 2007; Smolka et al. 2007). From the findings

summarized above, it is clear that the DDR is complex at the molecular level. This complexity reflects not only its importance for survival but also the need for a highly specific, modulated response. Identifying targets of the DDR is critical to understanding how and to what extent the DDR regulate cellular pathways in response to DNA damage. However, the definition of lesion and genotoxic dose specificity in relation to the final biological outcomes are essential for identifying specific substrates that are regulated by the DDR and that can be targets of therapy associated with DDR-dependent syndromes and cancer. An important goal in the future is to understand the direct relationship between the DDR and the diverse areas of biology that it regulates through PTMs and the consequences of these relationships to the integrity of the genome.

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