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Regulation of translesion DNA synthesis: Posttranslational modification of lysine residues in key proteins

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ABSTRACT

Posttranslational modification of proteins often controls various aspects of their cellular function. Indeed, over the past decade or so, it has been discovered that posttranslational modification of lysine residues plays a major role in regulating translesion DNA synthesis (TLS) and perhaps the most appreciated lysine modification is that of ubiquitination. Much of the recent interest in ubiquitination stems from the fact that proliferating cell nuclear antigen (PCNA) was previously shown to be specifically ubiquitinated at K164 and that such ubiquitination plays a key role in regulating TLS. In addition, TLS polymerases themselves are now known to be ubiquitinated. In the case of human polymerase η, ubiquitination at four lysine residues in its C-terminus appears to regulate its ability to interact with PCNA and modulate TLS. Within the past few years, advances in global proteomic research have revealed that many proteins involved in TLS are, in fact, subject to a previously underappreciated number of lysine modifications. In this review, we will summarize the known lysine modifications of several key proteins involved in TLS; PCNA and Y-family polymerases η, ι, κ and Rev1 and we will discuss the potential regulatory effects of such modification in controlling TLS *in vivo*.

1. Introduction

Posttranslational modifications (PTMs) of proteins by attaching different functional groups to amino acids widens the target protein's range of function and provides additional mechanisms by which the modified protein can be regulated. For example, PTMs can control a protein's activity by influencing its ability to interact with protein-partners, alter its enzymatic activity, subcellular localization, and change the stability of the protein. Of all the experimentally identified PTMs in mammals, serine phosphorylation is the most frequent modification followed by lysine, which represents over 15% of all experimentally identified amino acid modifications (calculation based on data from [1]). Lysine can be modified in a variety of ways including, but not limited to: ubiquitination, ubiquitin-like protein (UBL) modification *e.g.* SUMOylation, ISGylation, neddylation, FATylation and other lysine modifications such as acetylation, methylation, butyrylation, crotonylation, glycation, malonylation, phosphoglycerylation, propionylation, succinylation, myristoylation [1–4].

Eukaryotic cells have evolved a plethora of mechanisms in order to protect genome stability by removing DNA lesions, or preventing their conversion into permanent mutations [5]. Importantly, due to partially overlapping functions of some of these pathways, or time and conditional cellular requirements, their actions need to be precisely controlled. Recent studies in the DNA repair field have accumulated evidence of an ever expanding role of ubiquitination in regulating diverse DNA repair mechanisms and pathways involved in genomic stability maintenance (reviewed in [6]). Ubiquitin- and ubiquitin-like-dependent signaling processes have an important function in controlling cellular responses to DNA damage by navigating through the range of DNA damage repair, or tolerance mechanisms (reviewed in [6–10]). The majority of DNA lesions are repaired by one of the specialized DNA repair pathways; however the repair processes can be slow and incomplete and as a consequence a number of DNA lesions remain in the template DNA. This causes a severe problem, especially during the S-phase of the cell cycle, when DNA is replicated, because efficient and accurate classical DNA polymerases are blocked at DNA lesions. At this critical juncture, distinct mechanisms are required to temporarily

Abbreviations: TLS, translesion synthesis; PCNA, proliferating cell nuclear antigen; pol, polymerase; PIP, PCNA-interacting peptide; RIR, Rev1-interacting region; UBM, ubiquitin binding motif; UBZ, ubiquitin binding zinc motif; polι, DNA polymerase iota; polη, DNA polymerase eta; polκ, DNA polymerase kappa; UBL, ubiquitin-like protein; PTM, posttranslational modification; PRR, post replication DNA repair.

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tolerate cellular DNA damage, thereby avoiding the permanent block to the replication fork and the threat of cell cycle arrest. Lesion tolerance can be achieved in two different ways; one *via* a damage avoidance pathway using the information from the undamaged sister chromatid as a template for replication of the damaged DNA region, or *via* translesion synthesis (TLS), which employs specialized DNA polymerases to synthesize past the lesion.

Over the past dozen years, it has become evident that modification of lysine residues through the covalent linkage of ubiquitin, or ubiquitin-like proteins, plays a central role in controlling both DNA damage avoidance mechanisms and TLS. This review will attempt to summarize the known sites and cellular effects of ubiquitination of several key proteins involved in TLS. We will recap the individually discovered and experimentally confirmed sites of ubiquitination and ubiquitin-like modifications of TLS proteins and combine them with recent data derived from multiple proteome-wide approaches that reveal a hitherto underappreciated extent of lysine ubiquitination of many of the TLS proteins.

2. Types of lysine modifications

2.1. Ubiquitination

In eukaryotic cells, ubiquitination is involved in the regulation of almost all cellular processes, including cell division, membrane transport, signal transduction, DNA repair, endocytosis, inflammatory signaling, apoptosis, *etc.* [11–14]. It has been estimated that roughly 10% of human genes encode for proteins involved in ubiquitin metabolism [15]. The malfunction of ubiquitination processes and ubiquitin-mediated proteolysis has been implicated in various pathologies, including neurodegenerative disorders, inflammatory diseases and cancers [16–19]. Due to their important cellular functions, ubiquitination pathways are significant targets for therapeutics [20,21].

Protein ubiquitination is a dynamic and reversible process where a three-step enzymatic cascade conjugates a small, regulatory protein, ubiquitin, to a specific lysine residue in a target protein [22]. Initially, one of the ubiquitin-activating enzymes (E1s) forms an ATP-dependent thioester bond with ubiquitin. The activated ubiquitin is then transferred from the E1 enzyme to one of multiple ubiquitin-conjugating enzymes (E2s). E2 then transfers the activated ubiquitin to a protein substrate, either by itself, or with the help of one of the many ubiquitin ligases (E3s). Ubiquitin is linked through its C-terminal glycine residue to a specific internal lysine residue of the target protein. It has been also shown that in some proteins, ubiquitin can be attached to the N-terminus of the protein and in rare cases to a serine, threonine or cysteine residue [23–25]. Monoubiquitinated substrates can undergo further ubiquitination [26–28]. There are seven lysine residues in ubiquitin; K6, K11, K27, K29, K33, K48 and K63; all of them can be involved in polyubiquitin chain assembly. Additionally linear N-terminal polyubiquitin chains can also be formed [29]. Depending on the type of ubiquitin-chain linkage, polyubiquitinated proteins might be destined for degradation by the 26S proteasome in an ATP-dependent manner or alternatively, polyubiquitination might provide a signal for distinct cellular processes such as the inflammatory response or DNA repair [10]. Chains that are linked through K48 are the principal signal for degradation by the proteasome [30,31]. Recent studies, based on mass spectrometry have shown that homogeneous chains consisting of K29, K11, K27 and K6-linkages, heterogeneous chains with mixed lysine linkages, as well as multiple nearby monoubiquitination and, in cases of substrates up to 150 amino acids, even monoubiquitination can promote proteasomal degradation [32,33]. Chain elongation of ubiquitinated substrates is mediated *via* another class of ubiquitin ligases, E4s [34,35]. Ubiquitination can be reversed through the activity of de-ubiquitinating enzymes (DUBs), which primarily disassemble polyubiquitin chains before protein degradation, but will also cleave off a single ubiquitin moiety, or a polyubiquitin chain to regulate protein functionality [36].

2.2. Ubiquitin-like posttranslational modification

Besides ubiquitin, at least 10 different ubiquitin-like proteins (UBLs) exist in mammals (reviewed in [37,38]) with SUMO, NEDD8 and ISG15 being the best known. UBL modifiers, similar to ubiquitin, form an isopeptide bond between their C-terminal glycine and lysine residues of the substrate [38]. UBLs often have low sequence homology, but share a similar three-dimensional structure [38]. Posttranslational modification with UBL proteins can alter cellular function, stability, interactions with protein partners, or subcellular localization of the target protein [37,39]. Protein modification by UBLs follows the same three-step cascade similar to ubiquitination in that it is catalyzed by sets of analogous activation (E1), conjugation (E2s) and ligation (E3s) enzymes and can be reversed by deconjugating enzymes [40].

SUMO (Small Ubiquitin-like MOdifier) is the most studied UBL modifier and is expressed in all eukaryotes, mainly as a single variant. However in human cells there are four different paralogs (SUMO1–4), representing various homology, expression levels and substrate preferences. Many proteins interacting with a SUMOlyated substrate possess specific SIM domains (from SUMO-interaction motif) [41]. SUMOylation of a target protein can influence the protein degradation, signal transduction, localization, transcription activation, cell cycle, chromatin organization, DNA repair and other functions (reviewed in [42]). Dysfunction of SUMOylation can lead to neurodegenerative diseases, heart defects, diabetes or cancer [42–45].

One ubiquitin-like molecule, ISG15 (the interferon-stimulated gene 15), has a primary sequence that consists of two domains with significant similarity to ubiquitin [46]. Interestingly, ISGylation shares some of the E2 and E3 enzymes used in ubiquitination and ISGylated proteins can also be targeted for degradation by the 20S proteasome [47,48]. ISG15 is only found in vertebrates. Type I interferon, viral infection, lipopolysaccharides and some types of genotoxic stress can rapidly induce ISG15 conjugation [49,50] and it has been shown that enhanced ISGylation correlates with carcinogenesis [51].

Another example, NEDD8 (neural precursor cell-expressed developmentally downregulated-8), shares 60% identity and 80% homology with ubiquitin [52], and as a consequence, it can be incorporated into polyubiquitin chains by the E2 and E3 ubiquitin-conjugating enzymes [53]. The best characterized substrates known to be neddylated are cullins, scaffold proteins of SCF ubiquitin ligases (Skip-1, cullin, F-box) which regulate ubiquitination and proteasomal degradation of proteins involved in cell cycle control, transcriptional regulation, signal transduction [37,54]. Other, non-cullin neddylation substrates include proteins involved in RNA splicing, DNA replication and repair and proteasomal degradation [55].

3. Identifying ubiquitination and UBL modification sites

The identification of lysine residue(s) to which ubiquitin, or UBL proteins are conjugated, is important for understanding its biological significance. Locating ubiquitination, or UBL sites, can be performed experimentally, using conventional approaches, such as site-directed mutagenesis of a potentially modified residue [56,57], or by using antibodies against ubiquitin, or UBL proteins [58,59]. Recently, however, high-throughput methods and