DNA Repair 8 (2009) 544–556

Contents lists available at ScienceDirect

DNA Repair

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

Review

# Ubiquitin-binding domains and their role in the DNA damage response

Kay Hofmann*

*Bioinformatics Group, Miltenyi Biotec GmbH, Friedrich-Ebert-Straße 68, 51429 Bergisch-Gladbach, Germany*

ARTICLE INFO

*Article history:*
Available online 12 February 2009

*Keywords:*
Ubiquitin
UBA
UBM
UBZ
PCNA
H2AX
Translesion synthesis
Histone modification

ABSTRACT

The modification of eukaryotic proteins by covalent attachment of ubiquitin is a versatile signaling event with a wide range of possible consequences. Canonical poly-ubiquitination by Lys-48 linked chains usually destines a protein for degradation by the proteasome. By contrast, attachment of a single ubiquitin or ubiquitin chains linked through Lys-63 or Lys-6 serves a non-proteolytic role. Over the last years, evidence has accumulated that several nuclear proteins become ubiquitinated in response to DNA damage. Typically, these proteins carry mono-ubiquitin or non-classical ubiquitin chains and are localized close to the site of DNA damage. Of particular interest are PCNA and the variant histone H2AX, two key proteins whose ubiquitination serves to recruit factors needed by the cell to cope with the damage. A prerequisite for docking effector proteins to the site of the lesion is the detection of a specific ubiquitin modification, a process that can be mediated by a range of dedicated ubiquitin-binding domains (UBDs). As the same types of ubiquitin modification are involved in entirely different processes, the recognition of the ubiquitin mark has to go along with the recognition of the modified protein. Thus, ubiquitin-binding domains gain their specificity through combination with other recognition domains and motifs. This review discusses ubiquitin-binding domains relevant to the DNA damage response, including their binding mode, their specificity, and their interdependence with other factors. For several repair pathways, current knowledge of the events downstream of the ubiquitin mark is sketchy. A closer look at orphan UBD proteins might lead to the identification of missing pieces in the DNA response puzzle.



**Contents**



* Tel.: +49 2204 8306 4327; fax: +49 2204 8306 5727.
*E-mail address:* kay.hofmann@miltenyibiotec.de.

1568-7864/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2009.01.003

## 1. Introduction

Since its discovery more than 25 years ago, the small protein ubiquitin has been found to be involved in nearly every important aspect of cell biology. In a process termed 'protein ubiquitination', the C-terminus of free ubiquitin is activated by a ubiquitin-activating enzyme (E1), passed on to a ubiquitin-conjugating enzyme (E2), and finally transferred onto a lysine residue of a target protein with the help of a ubiquitin ligase (E3). Besides the modification of heterologous proteins, the targeted lysine can also reside within a second ubiquitin molecule, resulting in the formation of poly-ubiquitin chains. Originally, protein ubiquitination was thought to universally mark the target proteins for degradation by the proteasome [1]. By now, it is understood that proteasomal targeting is only one facet of the ubiquitin system, and is selectively mediated by poly-ubiquitin chains linked through lysine-48 of ubiquitin, the 'K48 chains'. Other, non-proteasomal, ubiquitin signals are based on mono-ubiquitination or other types of poly-ubiquitin chains, including those linked through K63 and K6 [2].

Nowadays, the ubiquitin system can be viewed as a versatile intracellular signaling system with a complexity rivaling that of protein kinase signaling. Analogous to other signal transductions systems, there are mechanisms in place for generating, sensing, and terminating any of the ubiquitin-based signals. The human genome encodes about 40 different E2 enzymes and more than 500 different E3 ligases, most of which are probably actively involved in protein ubiquitination. On the signal termination side, mammals command about 90 different deubiquitinating enzymes (DUBs) for cleaving specific poly-ubiquitin chains, or entirely removing ubiquitin from the target proteins. The number of ubiquitin recognition components is harder to estimate, as they belong to a variety of different protein classes. In the vast majority of cases, ubiquitin receptor proteins have a modular architecture where the specific recognition of the ubiquitin signal is performed by dedicated ubiquitin-binding domains (UBDs).

Gene products involved in several DNA damage response and repair pathways contain hallmarks of ubiquitin-conjugating, -ligating, or -recognizing proteins. Over the last years, a number of proteins were found to be ubiquitinated upon irradiation or treatment with DNA-damaging agents. Among those proteins are the proliferating cell nuclear antigen PCNA [3], the core histone H2A and its variant H2AX [4], the 9-1-1 complex [5], the Fanconi-pathway proteins FANCD2 and FANCI [6], and the replication factor Rfc2 [7]. At least in the case of PCNA and H2AX, the ubiquitinated versions form foci at the damage site and are instrumental for recruiting downstream proteins for resolving the situation. The ubiquitin marks at the damage site are based on mono-ubiquitin or K63-chains, and the downstream proteins have to recognize these modifications over a background of constitutively ubiquitinated proteins with all kinds of linkage types.

## 2. Ubiquitin-binding domains and proteins

In the majority of cases, ubiquitin and ubiquitinated proteins are recognized by dedicated ubiquitin-binding domains (UBDs), which form autonomous folding units within the ubiquitin receptor proteins. Ubiquitin-binding domains can be classified into a number of different families, whose members share sequence and structural similarity only within the families, but not between them. Currently, more than ten such families are known, each of them with multiple members in a given genome. There are a number of comprehensive reviews on the various classes of ubiquitin-binding domains and their properties [8–14]. This review focuses on those domain types that are relevant in the context of the cellular DNA damage response (Table 1).

### 2.1. Shared features

Typical ubiquitin-binding domains have been initially discovered in bioinformatical sequence database searches, where they appear as regions of locally confined sequence similarity shared by multiple proteins known or suspected to bind to ubiquitin [12]. Like other functional protein domains, the dedicated UBDs can fold independently of the rest of the host protein, and can – at least to a certain degree – also function in isolation. The bioinformatical prediction of the domain boundaries and of the key conserved

**Table 1**
Relevant types of ubiquitin-binding domains (UBDs). Proteins shown in brackets are bioinformatical predictions; so far no ubiquitin-binding has been shown.

| Domain | Literature references | | Members involved in DNA damage | | |
|---|---|---|---|---|---|
| | Discovery | Binding mode | Yeast | Human | Pathway |
| UBA | [22] | [15,21,133] | Rad23<br>Rad23, Dsk2, Ddi1 | hHR23a,b<br>hHR23a,b Ubiquilin-1 to 5 | Excision repair<br>Proteolysis |
| UIM | [33] | [42,134,135] | –<br>Rpn10 | RAP80<br>S5a | DSB response<br>Proteolysis |
| UEV | [44,45] | [51,136] | Mms2 | Mms2 | K63 conjugation |
| UBM | [20] | – | Rev1<br>–<br>Rad2 | Rev1<br>Pol ι<br>XPG | Translesion bypass<br>Translesion bypass<br>Excision repair |
| UBZ3 | [20] | [58] | Rad30 | Pol η | Translesion bypass |
| UBZ4 | [20] | – | –<br>Mgs1<br>Rad18<br>–<br>[Pso2] | Pol κ<br>WRNIP1<br>Rad18<br>[RAP80]<br>[Artemis] | Translesion bypass<br>Genome stability<br>PCNA modification<br>DSB response<br>DSB response |