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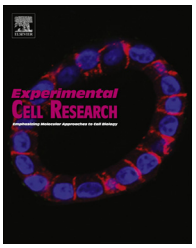

Review Article

Should I stay or should I go: VCP/p97-mediated chromatin extraction in the DNA damage response

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ABSTRACT

The ordered assembly of DNA repair factors on chromatin has been studied in great detail, whereas we are only beginning to realize that selective extraction of proteins from chromatin plays a central role in the DNA damage response. Interestingly, the protein modifier ubiquitin not only regulates the well-documented recruitment of repair proteins, but also governs the temporally and spatially controlled extraction of proteins from DNA lesions. The facilitator of protein extraction is the ubiquitin-dependent ATPase valosin-containing protein (VCP)/p97 complex, which, through its segregase activity, directly extracts ubiquitylated proteins from chromatin. In this review, we summarize recent studies that uncovered this important role of VCP/p97 in the cellular response to genomic insults and discuss how ubiquitin regulates two intuitively counteracting activities at sites of DNA damage.

Contents

Abbreviations: CPDs, Cyclobutane pyrimidine dimers; DSBs, DNA double-strand breaks; ER, endoplasmic reticulum; ERAD, ER-associated degradation; HR, homologous recombination; 6-4PPs, pyrimidine-pyrimidone [6-4] photoproducts; IR, ionizing radiation; UBD, ubiquitin-binding domain; UV, ultraviolet light; VCP, valosin-containing protein

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General introduction

Knowing when to leave a party is often equally important as showing up on time. The same holds true for the protein crowd that gathers at sites of DNA damage. However, in sharp contrast to the wealth of data on a rapidly expanding number of proteins that is recruited to sites of DNA damage, researchers have only started to explore the mechanisms that control the selective removal of DNA damage response (DDR) proteins. The coordinated removal of proteins from DNA lesions can be important to make space for the binding of additional DNA repair proteins, to remove proteins whose activity may be inhibitory to DNA repair, to restrict DNA repair or signaling pathways once these are activated, or to restore the pre-damage chromatin status once the lesion has been repaired. Specific cues play a role in orchestrating the timely entry and exit of individual proteins at DNA lesions. Ubiquitylation, by virtue of its versatile nature, functions as a signal in these events and plays an important role in regulating the assembly as well as the disassembly of proteins at DNA lesions.

The ubiquitin-dependent recruitment of DDR proteins is largely accomplished by the association of specific ubiquitin-binding domains (UBDs), which reside within the recruited proteins themselves or in one of their binding partners, with chromatin-associated ubiquitin conjugates that are synthesized in a tightly regulated fashion at sites of DNA damage [1]. The removal of DDR proteins from chromatin, on the other hand, is more mechanical as it requires an enzymatic activity that pulls proteins from the chromatin fiber resulting in their extraction. Recent studies have revealed that the ubiquitin-specific segregase valosin-containing protein (VCP)/p97 (known as CDC48 in yeast or CDC-48 in nematodes) plays a central role in selecting and removing ubiquitylated proteins from sites of DNA damage.

VCP/p97 is a highly abundant, chaperone-like monohexameric complex that belongs to the family of AAA (*ATPases-associated with various cellular activities*) ATPases [2] and associates with a panel of specific adaptor proteins, many of which contain UBDs [3]. VCP/p97 is related to the AAA-ATPases that are located at the entrance of the proteasome and unfold and translocate substrates into the proteolytic chamber [4]. Correspondingly, it is believed that the ATPase activity of VCP/p97 bestows this complex with a pulling force that enables it to segregate ubiquitylated proteins from their environment, such as multiprotein complexes, membranes, or chromatin [5]. Ubiquitin-dependent extraction from chromatin, which was first discovered for the cell cycle-dependent removal of Aurora B from chromatin [6], has similarities to the most intensively studied role of VCP/p97, namely the extraction of misfolded proteins that reside in the endoplasmic reticulum (ER). Such proteins are subsequently degraded by the proteasome [7] in a process known as ER-associated degradation (ERAD) [8]. In addition to its well-established role in ERAD, several recent studies have uncovered an important role of VCP/p97 in DNA damage signaling and repair. In the following sections, we will highlight the versatile role of VCP/p97-driven chromatin extraction in regulating the DNA damage response at multiple levels.

VCP/P97-mediated extraction at photolesions

Transcription-coupled nucleotide excision repair (NER)

Ultraviolet light (UV)-induced DNA lesions, such as pyrimidine-pyrimidone [6-4] photoproducts (6-4PPs) and cyclobutane pyrimidine dimers (CPDs), form a potential treat for the integrity of the genome and are removed by nucleotide excision repair (NER), a versatile DNA repair pathway that can efficiently eliminate a variety of structurally unrelated lesions [9]. RNA polymerase II (RNA pol II) complexes that are stalled at photolesions initiate DNA repair by transcriptional-coupled NER (TC-NER), which selectively removes lesions from actively transcribed genes. The stalling of RNA pol II is presumably followed by backtracking of the complex to expose the lesion and make it accessible to NER proteins. The displacement of the stalled RNA pol II is subsequently followed by the selective destruction of its largest subunit, Rpb1, by ubiquitin-dependent proteasomal degradation [10] (Fig. 1A). In yeast, UV-induced ubiquitylation of Rpb1 is mediated by the HECT ubiquitin ligase Rsp5 or the CUL3-based CRL3 complex [11] while in human cells the Rsp5-related NEDD4 regulates the UV-induced degradation of Rpb1 [12], suggesting a conserved role of Rpb1 ubiquitylation in NER.

A quantitative comparison of proteins that were bound to proteasomes in yeast expressing either wild-type CDC48 or a mutant CDC48 that is defective in supporting ubiquitin-dependent degradation revealed that the ATPase activity of CDC48 is required for an efficient interaction between Rpb1 and the 26S proteasome [13]. While this interaction is already present in unchallenged cells, suggesting a role for CDC48 in the constitutive degradation of Rpb1, it appeared that CDC48 is also required for UV-induced degradation of Rpb1. Interestingly, besides the UFD1/NPL4 adaptor of CDC48, which links this complex to ubiquitin-dependent proteasomal degradation, this activity also required the less well-understood adaptors UBX4 and UBX5 [13]. These adaptors appear to be specific for recruiting CRL3-ubiquitylated Rpb1 given that deletion of CUL3 reduces the levels of ubiquitylated Rpb1 [13]. Consistent with the degradation of Rpb1 after UV, CUL-based ubiquitin ligases typically synthesize ubiquitin chains that are linked by lysine 48 in ubiquitin (K48 ubiquitin chains), the canonical signal for proteasomal degradation, whereas Rsp5 provides substrates with monoubiquitin or K63-linked ubiquitin chains (K63 ubiquitin chains) [14], which fulfill non-proteolytic roles [15]. Together these data show that CDC48 is implicated in facilitating the UV-induced proteasomal degradation of Rpb1 in yeast (Fig. 1A). Whether VCP/p97 is involved in the extraction and degradation of RNA pol II in human cells remains to be established. Such a scenario seems plausible given that at least a small fraction of the VCP/p97 molecules is

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