Putting the Proteasome at the End: An Emerging Role for the Ubiquitin-Proteasome System in DNA Double Strand Break Repair

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Abstract
The proteasome is best known for its function in the orderly degradation and turnover of proteins, and in this capacity is important in a wide variety of cellular processes from protein quality control to cell cycle regulation. Mounting evidence suggests that the proteasome is a rational therapeutic target in the treatment of cancer; in clinical trials, proteasome inhibitors have proven effective against hematological malignancies. On a cellular level, proteasome inhibitors sensitize the cell to DNA damage, suggesting a link between the proteasome and DNA repair pathways. The proteasome's involvement in cellular response to UV damage has been well established, but its role in other repair pathways, like homologous recombination, remains comparatively obscure. Here, we review recent research that implicates the proteasome as important in the repair of DNA double stranded breaks.

Introduction to the proteasome

The ubiquitin-proteasome system is responsible for the majority of protein degradation in the cell. It is responsible for recycling damaged and misfolded proteins, and for regulating diverse cellular processes by controlling the levels and localization of their effector proteins (reviewed in [1]). The eukaryotic 26s proteasome consists of two subcomplexes, the 20s core and the 19s regulatory particle. The cylindrical 20s core is where the protease activity takes place, with the 19s cap controlling the recruitment, processing, and access of the proteins targeted for degradation [2]. These proteins are marked for destruction with ubiquitin tags (Ub), which are covalently attached to the target protein by a complex cascade of ubiquitin ligases that each recognize unique substrates and are precisely regulated, conferring much of the specificity of the Ub-proteasome pathway [3]. Polyubiquitin chains linked via lysine 48 (K48) are the primary signal flag for the proteasome, although recent research suggests that this rule is not as strict as once thought and that other ubiquitin chains, or even mixed linkages, might play a role in the ubiquitin-proteasome pathway [4].

DNA double stranded breaks

DNA double stranded breaks (DSB) are one of the most toxic cellular lesions: even one unrepaired DSB can lead to cell death. Inducing DSBs has, then, become a major strategy in cancer treatment. Many endogenous and exogenous agents can inflict DSBs, including ionizing radiation, radiomimetic agents, topoisomerase inhibitors, free radicals, and even the collapse of replication forks during normal S phase. Therefore, the cell has evolved a number of mechanisms to repair of this type of damage [5]. The two major pathways are

nonhomologous end joining (NHEJ) and homologous recombination (HR) [reviewed in [6]). The most high-fidelity type of repair is homologous recombination (HR), which uses the sister chromatid as a template in order to ensure seamless resolution of the break, in comparison to NHEJ which ligates DNA ends without sequence specificity [7]. Briefly, upon detection of the double stranded break, exonucleases resect the ends to form a long stretch of single stranded DNA, which is coated by the single stranded binding protein RPA. With help from proteins like BRCA2, RAD51 replaces RPA, yielding a protein:DNA structure that can search for the homologous sequence and initiate recombination [6] (Figure 1). Unwanted or misregulated HR, however, can lead to harmful gene conversion or mitotic crossover events, so HR must be tightly regulated [8].

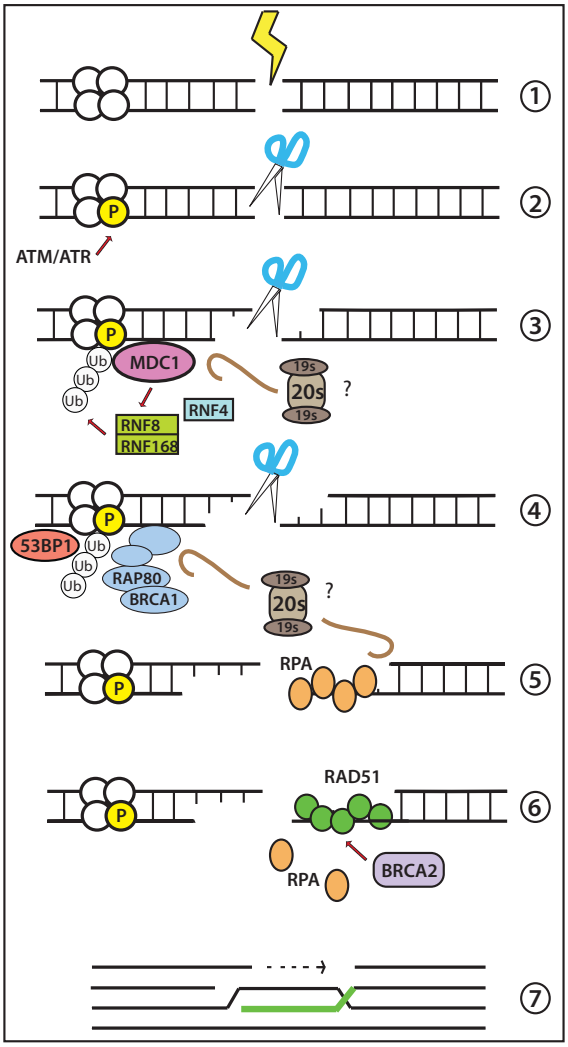

Figure 1: Schematic of Cellular Response to Double Stranded Breaks. 1) Upon the induction of double stranded breaks, a damage response cascade is triggered, beginning with the activation of the ATM and ATR kinases. 2) ATM and ATR phosphorylate numerous targets, with phosphorylated histone H2AX (δH2AX) functioning as a major recruitment mark. At this step, nucleases responsible for end resection, like Mre11, are also recruited to the break. 3) Early responders such as MDC1 are recruited to the δH2AX mark, and can support the recruitment of ubiquitin ligases like RNF8, RNF168, and RNF4. 4) The polyubiquitin marks created by these ligases recruit downstream pathway members, like 53BP1 and the RAP80-BRCA1-BARD1-Abraxas complex, which mediate the access of DDR proteins to the lesion and regulate strand resection. 5) The resected 3' flap is coated with the ssDNA binding protein RPA. 6) With help from BRCA2, RPA is replaced by the recombinogenic protein RAD51. 7) The RAD51-coated filament can initiate HR, functioning in homology search, D-loop formation, and strand invasion. Points of putative proteasome involvement are shown; the hooked line represents the removal of proteins from chromatin.

Clinical research implicates the proteasome in DNA damage response

Proteasome inhibitors are currently a hot topic in chemotherapy, with bortezomib (commercially Velcade^{TM}) proving to be

successful in the treatment of numerous system and DSB repair, with proteasome hematological malignancies including mantle functional link between the Ub-proteasome cell lymphoma and multiple myeloma [9]. Previous studies into drug combinations in cancer treatment have further suggested a inhibitors increasing the effectiveness of xenotoxic agents, like epirubicin, that are known to cause DSBs [10]. However, this sort of synthetic lethality does not guarantee a direct link between the two processes at a molecular level; therefore, understanding the mechanism of proteasome action at DSBs will be of utmost importance to exploiting this therapeutic avenue.

The proteasome in DSB repair

The Ub-proteasome system certainly has an effect on the ability of the cell to carry out DSB repair. Some of this effect can be attributed to the indirect consequence of ubiquitin starvation. When the inhibited proteasome cannot process proteins that have been covalently tagged with Ub, this reduces the overall available pool of ubiquitin, and thereby mutes ubiquitin-dependent signalling cascades in DNA damage response (DDR) [11]. Several proteasomal subunits are dynamically modified by both ubiquitylation and phosphorylation following treatment by ionizing radiation or radiomimetic drugs, suggesting that the modulation of proteasome activity is functionally important in DDR [12-14]. Proteasome subunits are also recruited to the sites of DSBs, which is again consistent with a direct effect on repair. In yeast, the proteasomal subunit Sem1 is recruited to HO endonuclease-induced breaks [15]. In mammalian cells, a number of both 20s (PSMA6, PSMA3) and 19s (PSMD7, PSMD4) subunits, as well as the nuclear proteasome activator PA28γ, have been shown to localize to laser stripes, which induce localized double strand breaks [13, 16], and the 19S subunits POH1 and PSMC5 are recruited to IR-induced foci [17]. Taken together, these data strongly suggest that the proteasome is mechanistically involved in the faithful completion of DSB repair.

Some of the first evidence for direct proteasomal involvement in DSB repair was an observation that treating cells with proteasome inhibitors causes persistent post-damage foci of MDC1, a protein that is recruited to DSBs early and regulates subsequent events in HR [18]. When MDC1 foci are not dissolved, it severely impairs the recruitment of BRCA1, leading to a reduction in DSB repair efficiency [18]. A more recent paper implicates the ubiquitin ligase RNF4 as a critical factor in damage-dependent recruitment of the 19s subunit PSMD4 to laser stripes: when RNF4 or PSMD4 is depleted by RNAi, persistent MDC1 occupancy at sites of DSBs is also present [16]. The depletion of RNF4 additionally causes increased occupancy of RPA1, a single stranded binding protein that precedes and is replaced by RAD51 during HR, at sites of damage, although the proteasomal dependence of this transaction remains unclear.

When either of the proteasome activators PA200 or PA28γ is depleted by RNAi, cells become hypersensitive to ionizing radiation and radiomimetic drugs, respectively, indicative of malfunction in DSB repair, [19]. With PA28γ depletion, early recruitment events in DSB repair like the phosphorylation of histone H2AX and foci formation by MDC1, 53BP1 and BRCA1 are unperturbed, but the

duration of these foci is extended in the PA28γ knockdown cells. The balance between repair by NHEJ and HR in these cells is disturbed, with a dramatic elevation in the levels of HR, which may be due to increased aggregation of the HR protein RAD51 around sites of damage [13]. Recent work also implicates the deubiquitylating enzyme and proteasome component POH1 in DSB repair. In order to properly process a DSB during HR, 53BP1 and the ubiquitin-binding protein RAP80 must be cleared from the DNA ends to allow for the early steps of HR, resection and strand-coating by RPA [17, 20, 21]. In the absence of POH1, 53BP1 and RAP80 binding proximal to the break is increased, RPA loading is decreased, and HR is impaired. In this case, the proteasome may work to displace proteins by removing the ubiquitin conjugates that they bind to, rather than acting directly on the proteins themselves.

Conclusions

The data reviewed here share a common theme: that the proteasome is required for clearing proteins from the chromatin around DSBs in order to facilitate normal processing and repair, a proposed role consistent with its familiar work in protein turnover. What is interesting is that the results of each of these studies are in some ways contradictory. Depletion of PA28γ causes an increase in HR, while depletion of POH1 leads to a decrease. Similarly, depletion of PA28γ causes increased loading of RAD51 onto chromatin at sites of damage, while knocking down RNF4 and thereby impeding access of PSMD4 to DNA leads to persistent RPA occupancy, which is refractory to RAD51 accumulation. The apparent conflict between these data may mean that the proteasome is involved in multiple steps during the repair of DSB, with different regulatory subunits and interacting proteins coordinating a complicated choreography of protein turnover at sites of damage. In that sense, global proteasome inhibitors may give us only a blurred vision of the process.

Taken together, the data covered in this review strongly support a role for the proteasome in DSB repair, but many questions remain. Research thus far has focused mainly on HR, but is there proteasomal regulation of NHEJ or of the pathway choice between NHEJ and HR? More mechanistically, is the classical proteolytic activity of the proteasome vital to its role in DSB repair, or can members of the 19s cap act in a degradation-independent manner to regulate complex disassembly? Which subunits are functionally crucial and which are just "along for the ride"? Can the relatively bulky proteasome directly interact with chromatin or are mediators required? For example, recent work on the AAA protein VCP/p97 has suggested that ATP-dependent remodeling activity at damage foci may be required to extract proteins before they can be directed towards the proteasome [22]. Dissecting the roles of different subunits in directing proteasome activity at different phases of repair will provide a clearer window into the interface between the proteasome and DNA repair, and may even offer more specific therapeutic targets for future clinical study.

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