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Role of proteasomes in transcription and their regulation by covalent modifications

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1. ABSTRACT

The 26S proteasome is an abundant multi-subunit complex, which, in addition to lysosomes, represents a major cellular "protein degradation factory". The proteasome complex possesses protease, ATPase/helicase, and RNAse enzymatic activities, which are used by the latter to regulate various physiological processes. Recent findings have revealed an important role of proteasomes in transcriptional regulation. Although proteasomes are well documented to undergo various post-translational modifications, little is known about their functional significance, in particular in the process of gene regulation in response to various forms of stress. Here, we review the data on the role of proteasomes in gene regulation and their post-translational modifications as well as discuss potential mechanisms by which proteasomal activity may be regulated by genotoxic stress.

2. INTRODUCTION

The 26S proteasome (dubbed for its sedimentation velocity) is an abundant multi-subunit protein complex, which is present in both the nucleus and cytoplasm of all eukaryotic cells. The major function of the proteasome is to degrade polyubiquitylated proteins by limited and controlled proteolysis, thus regulating the majority of the vital cellular processes (1, 2). The 26S proteasome consists of a barrel-shaped 20S catalytic core complex and two regulatory 19S complexes (3). In turn, each of the 19S complexes contains 17 subunits of which 6 possess ATPase activity (2). Both 19S subunits (referred to as the "base" and the "lid") participate in recognition and unfolding of ubiquitylated substrates and thus govern the proteolytic activity of the 20S core subunit. The 20S core complex itself is composed of four stacked seven-chain rings. The two outer rings are made of α-type subunits and

the two inner rings are made of β-type subunits (1, 4). Only the β1, β2, and β5 subunits are proteolytically active, featuring caspase-like, trypsin-like, and chymotrypsin-like peptidase activities, respectively (5, 6).

3.1. Proteasome-dependent regulation of transcription

Perhaps not surprisingly, the proteasomes, due to their enzymatic activity and high cellular abundance (approximately 1% of all cellular proteins), were found to regulate a wide variety of cellular processes, including gene expression. Genome-wide chromatin immunoprecipitation analysis has revealed that the proteasome components associate with the majority of yeast genes. In many cases this association positively correlated with gene transcription and the presence of RNA polymerase II on these genes (7). However, several hundred genes were cross-linked exclusively either to the 20S or 19S complexes, but not to both. On a related note, two types of proteasomes, both associated with genomic DNA and yet carrying distinct functions, were found to exist in embryonic stem (ES) cells (8). Specifically, the Rpn12 and Rpt3-containing complexes promoted the association of transcription factors with promoters of certain genes, whereas the intact 26S proteasomes inhibited spurious transcription from the intergenic regions (8, 9). Collectively, these data imply that the 20S and 19S sub-complexes may exert important regulatory functions outside of the context of the intact 26S proteasome.

Proteasomes have been found to affect each step of the transcription process: initiation, elongation, and termination. Importantly, the proteasomes utilize both their proteolytic and non-proteolytic activities to control all of the above processes.

3.1.1. Role of proteasomes in transcriptional initiation

Several reports have demonstrated the positive effects of the two non-proteolytic proteasome subunits of the 19S complex, SUG1/Rpt6/PSMC5 and SUG2/Rpt4/PSMC6, on binding of the Gal4 transcription activator to chromatin and increased recruitment of components of the transcriptional machinery, including a subunit of yeast FACT (Cdc68/Pob3), TFIID, TFIIH, and the RNA polymerase II holoenzyme (10, 11).

Interestingly, both SUG1 and SUG2 possess ATPase activity and SUG1 also harbors 3'-5' DNA-helicase activity connected with its ATPase function (12). Therefore, they may directly influence interactions between DNA and the transcription machinery and hence affect transcription activation and elongation.

Stable recruitment of the RNA Pol II holoenzyme complex to promoters and re-initiation of transcription also requires the proteolytic function of the proteasome. For example, the ability of yeast activator Gcn4 to recruit RNA Pol II to promoters is blocked upon chemical repression of proteolytic activity of the proteasome (13).

Since strong transcriptional activators are necessary only for a limited period of time, their activity and abundance are controlled by the proteasomes through ubiquitin-dependent degradation, a mechanism called "black widow" (14). The idea behind this regulatory mechanism is that monoubiquitylation constitutes a "licensing event" for activator function. However, addition of a single ubiquitin to a lysine epsilon amino group of an activator may result in subsequent ubiquitin chain growth, which would then attract the proteasome and result in activator destruction.

How does monoubiquitylation of the activator stimulate its activity? As any post-translational modification, monoubiquitylation may affect the activator in two ways: by facilitating its interaction with a specific effector protein, and/or by promoting other post-translational modifications thereby affecting the structure of the activator. While the latter possibility is yet to be explored, there is at least one report indicating that the monoubiquitylated LexA-VP16 chimeric activator increases the recruitment of P-TEFb elongation factor (15).

Recently, the Johnston group published an elegant study whereby they provided another plausible explanation to the "activating" role of monoubiquitylation of transcription activators (16). They showed that the 19S proteasomal ATPase activity is required for sustained de-stabilization of the Gal4-VP16 activator-promoter complex and that monoubiquitylation of the activator reversed this effect. It is not clear, however, whether this is a general phenomenon, or an activator-specific event (16, 17).

Activity of many important transcription factors, including NF-kappaB (see review of (18)), hormone receptors (19), tumor suppressors p53 and Rb (20-22), and the oncogene c-Myc (23, 24) are subject to regulation by ubiquitylation-dependent "licensing". In line with this notion, the 20S proteasome catalytic beta subunit LMP2 (Low Molecular mass Polypeptide 2) was shown to interact directly with the steroid receptor coactivator (SRC) protein. The recruitment of the 20S beta subunit LMP2 by SRC coactivator is necessary for cyclic assembly/disassembly of the estrogen receptor (ER)-regulated transcription complexes on the target genes (25).

The proteasome proteolytic activity is also critical for sustained glucocorticoid hormone response. The 20S subunit activity modulates glucocorticoid hormone receptor (GR)-dependent gene transcription at least in part by regulating turnover and recycling of receptor/transcriptional-DNA complexes (19).

It is important to note, however, that Rb, Egr-1, and several other activators and cell cycle regulators can also be degraded by the proteasome in an ubiquitin-independent manner through a direct interaction between the activator and the C8 (α7) subunit of the 20S proteasome core (26-28). Apparently, Mdm2, an E3 ligase specific for p53 and Rb, is instrumental in stabilization of these interactions since the ablation of or mutations in the central acidic domain of Mdm2 abrogates the association of the proteasome with the aforementioned activators (27, 29).

An alternative mechanism of ubiquitin-independent and 20S-dependent protein degradation is

mediated by NQO1, a NADH-dependent oxidase, which serves as a "gatekeeper" of the 20S proteasome (30, 31).

3.1.2. Role of proteasomes in chromatin remodeling

The contemporary dogma in the field of transcription postulates that in order to achieve physiological levels of transcription, genomic loci should undergo chromatin remodeling, which is governed by various combinations of histone modifications in concert with ATP-driven physical repositioning of the nucleosomes along the promoter/enhancer region. In accordance with its role in transcription, the regulatory components of the proteasome were reported to actively participate in the process of histone modifications (reviewed in (32)).

For instance, ATPases subunits SUG1 and SUG2 of the yeast 19S complex are necessary for methylation of histone H3 on lysine residues 4 and 79. These modification marks correspond to the active sites of transcription (33). Interestingly, binding of these subunits to the sites of active transcription depends on the ubiquitylation status of histone H2B (33, 34). Collins and Tansey have suggested that Sug1 and Sug2 subunits affect local chromatin structure to facilitate recruitment of the corresponding methyltransferases to their target sites (32). Whether these subunits of the 19S complex interact with DNA (due to their helicase activity) or histones (via chaperone activity) or both remains to be investigated as does the time of their appearance at gene regions.

In addition, Rpt6/Sug1 was reported to enhance both the promoter recruitment and HAT activity of the SAGA histone acetyltransferase complex. The latter, via H3-K14 acetylation, positively regulates transcription in various organisms from yeast to humans (35). Importantly, mutations in the ATPase domain of Rpt6 reduced SAGA/Gcn5-dependent H3 acetylation of target promoters in vivo. However, the molecular mechanism of this phenomenon is yet to be elucidated.

Lastly, histone modifications have also been shown to depend on the proteolytic activity of the proteasome. In the case of glucocorticoid receptor (GR)–induced transcription, inhibition of proteasome activity resulted in an increase of tri-methyl histone H3-K4 levels on the GR-regulated promoters. This local elevation of the H3-K4 tri-methylation level is likely due to stabilization and recruitment of a known histone methyltransferase, the Mixed Leukemia Lineage 2 (MLL2), by GR (19).

3.1.3. Role of proteasomes in transcriptional elongation and termination

The proteasome is also able to regulate transcription by affecting the process of elongation and termination. It has been shown that mechanistically, ATPases, particularly Sug1/Rpt6 and Sug2/Rpt4, stimulate promoter escape and elongation in a non-proteolytic fashion after being recruited to promoters through direct interactions with activation domains (10, 34). *In vivo*, yeast strains carrying mutant alleles of *SUG1* and *SUG2* (encoding the 19S components) exhibited phenotypes indicative of elongation defects. Addition of the immunopurified 19S complex was sufficient to repair the elongation defect imposed by a heat-inactivated temperature-sensitive Sug1 mutant in the transcription reaction *in vitro* (36). Moreover, the Sug1 protein co-immunoprecipitated with an elongation factor FACT, thus providing a mechanistic explanation for interaction between the 19S and RNA-Pol II complexes (10).

The proteolytic activity of 26S proteasomes is also necessary for accurate transcription termination because its inhibition increases the level of transcription through the termination site (37). Several reports describe selective targeting of the elongating form of the largest subunit of RNA polymerase II (RNA Pol II) by ubiquitin-mediated proteolysis in response to DNA damage. The evidence accumulated to date suggests that DNA damage blocks RNA Pol II progression (38-40). Consequently, damage-stalled RNA Pol II induces transcription-coupled DNA repair (TCR). However, if repair of the lesion by TCR fails, RNA Pol II undergoes Nedd4-dependent ubiquitylation and degradation (41). Thus, the proteasome-dependent proteolytic activity is also important for regulation of transcription-coupled DNA repair (42-44).

3.2. Proteasomes and their post-translational modifications

Obviously, active participation of the proteasome in various cellular processes, including transcription, requires a high degree of regulation. Proteasome regulation is achieved by several mechanisms: alteration of the proteasomal composition and/or post-translational modifications (See Table 1). For example, γ-interferon induces expression of several proteasomal subunits, β1i (LMP2), β2i (MECL1), and β5i (LMP7), which are homologous to the β1, β2, and β5 subunits of the constitutively expressed 20S complex. This leads to alteration of the proteasomal cleavage site preference and increased proteasomal production of antigenic peptides for MHC class I presentation (45, 46). γ-Interferon also affects the composition of the proteasome by promoting incorporation of an inducible 11S complex, designated as proteasome activator PA28, into the 26S complex (47). The latter substitutes for the 19S complex and is able to enhance production of the antigenic peptides for MHC class I presentation independently of the presence of immunosubunits in the 20S complex (46, 48).

Among post-translational modifications found in the proteasome, phosphorylation seems to play the central role in regulation of its activity and stability (49-52). For example, CKII-mediated phosphorylation of the C8 (α7) subunit of the 20S complex is critical for the stability and specificity of the 26S complex (53). γ-Interferon treatment decreased the level of C8 phosphorylation in parallel with attenuation of the cellular level of the 26S complex. On the contrary, the level of the PA28-containing immunoproteasomes increased due to instability of the 26S complex and hence the elevated exchange rate between the 19S and PA28 sub-complexes (47, 53).

Table 1. Post-translational modification of the proteasome subunits

Subunit	PTM, sites	Enzyme	Functional effects	Organism	Reference
	Phosphorylation				
α1	Ser~P; Tyr~P; Thr~P	n.d.	An increase of chymotrypsin- and caspase-like activities	*M. musculus*	58
α2	Ser~P, Tyr120~P	n.d.	An increase of chymotrypsin- and caspase-like activities, nuclear localization of the proteasome complex	*M. musculus, R. norvegicus*	58, 80, 81
α3	Ser~P, Ser248~P	PLK, CK2, and unknown	Binding of the regulatory proteins to the ends of the core particle; an increase of chymotrypsin- and caspase-like activities	*H. sapiens, M. musculus, C. albicans, R. norvegicus*	50, 51, 58, 82-84
α4, α5, α6	n.d.	CKI, CKII, and PKA	n.d.	*C. auratus, C. albicans, O. sativa, M. musculus*	50, 58, 85 86)
α7	Ser243~P, Ser250~P, Other sites	PKA, CK2, PLK, and unknown	Stabilization of the 26S proteasome complex via increased association between the 20S and 19S complexes; binding of the regulatory proteins to the ends of the core particle; an increase of chymotrypsin- and caspase-like activities	*H. sapiens, M. musculus, R. norvegicus, S. cerevisiae*	58, 87, 88 83, 84, 89
β2, β3, β7	Ser~P, Thr~P	PKA	An increase of chymotrypsin- and caspase-like activities	*M. musculus*	58
Rpt5	Ser9~P	n.d.	Substrate recognition and opening of a channel inside the 20S core particle	*H. sapiens*	52
Rpt6	n.d. Ser120~P	p45-kinase, PKA	Stabilization of the 26S proteasome complex via increased association between the 20S and 19S complexes; activation of the proteasome	*S. scrofa, H. sapiens*	54, 74, 90
PA28	Ser~P	n.d.	Binding to the 20S particle; proteolytic activity via conformational changes in the 11S complex	*H. sapiens*	51
	N-acetylation				
α1, α2, α3, α4, α7, β3, Rpt4, Rpt5, Rpt6, Rpn2, Rpn3, Rpn5, Rpn6, Rpn8		NatA/Nat1	n.d.	*S. cerevisiae*	61
α5, α6, β4, Rpt3, Rpn11		NatC/Mak3	n.d.	*S. cerevisiae*	64
	O-Glycosylation				
α2, α3, α4, α5, α6, α7, β2, β5, β7, Rpt2, Rpt3, Rpt4, Rpt6, Rpn3, Rpn5, Rpn6, Rpn12		OGT and unknown	Prevention of possible phosphorylation, extension of the protein's half-life; Inhibition of subunit-specific ATPase activity	*D. melanogaster H. sapiens;*	59, 60
	ADP-ribosylation				
20S complex		PARP	Activation of proteolytic activity	*H. sapiens*	63
	Myristoylation				
Rpt2			Activation of the 26S and 20S proteasomal complexes	*S. cerevisiae, M. musculus*	62, 64

Stability of the 26S proteasome is also regulated by the phosphorylation state of the Rpn16 subunit of 19S. Phosphorylation of this protein promotes association between the 19S and 20S complexes. This is achieved through the phosphorylation-dependent binding of the Rpn16 protein to the α2 subunit of the 20S complex (54).

Thompson and colleagues reported that phosphorylated S12 (Rpn8), a non-ATPase 19S regulatory subunit of the 26S proteasome, was found in six normal, but not four transformed, breast epithelial cell lines (55). Apparently, phosphorylation of S12 determines its cellular localization, since the phosphorylated form of S12 resides exclusively in the cytosol and is not associated with the 26S proteasome.

Relatively little is known about the identity of protein kinases responsible for proteasome subunits' phosphorylation. CKII (casein kinase II) was shown to co-purify with proteasomes in erythrocytes (56) and phosphorylate α7 subunit of the 20S complex (57). Recently, PKA (protein kinase A) association with murine cardiac proteasomes was described. It appears that PKA works in concert with PP2A (protein phosphatase 2A) to achieve fine-tuned regulation of proteasomal activity (58).

Several proteasome subunits were shown to be O-glycosylated by N-acetyl glucosamine. This post-translational modification may compete with phosphorylation for the same target serine and threonine residues. Several subunits of both 19S and 20S complexes were shown to be O-glycosylated in *Drosophila melanogaster* (59). Glycosylation of the Rpt2 subunit of the 19S complex decreased its ATPase activity and hence the ability of the 26S proteasome to efficiently proteolyze its substrates (60).

Other post-translational modifications found in the proteasome include poly-ADP ribosylation,

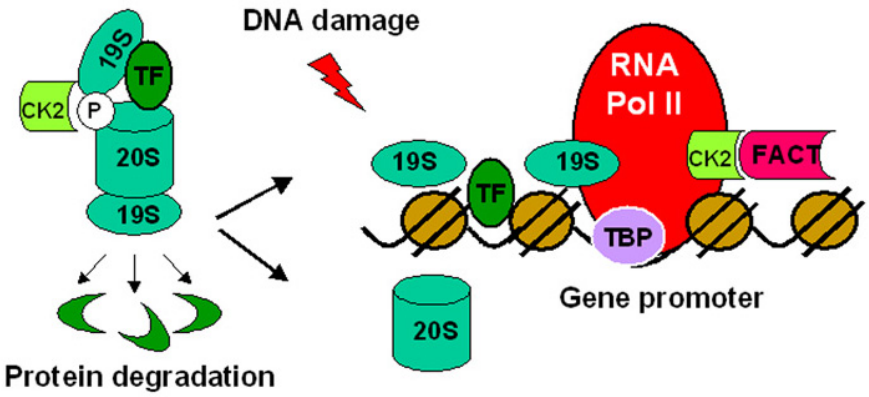

Figure 1. A model of DNA damage-induced dissociation of the 26S proteasome into the 20S and 19S sub-complexes. CK2 kinase phosphorylates α7 subunit of the 20S core complex to stabilize its binding to the 19S complex. Stable 26S proteasome degrades transcription factor, important for transcription of anti-apoptotic/DNA repair genes (left). Upon DNA damage, the specificity of CK2 changes due to its association with FACT (right). Consequently, the 26S proteasome dissociates and the regulatory ATPase-containing 19S complexes are recruited to gene promoters of anti-apoptotic/DNA repair genes to remodel chromatin and enhance transcription.

myristoylation and N-terminal acetylation (61-64). Both poly-ADP ribosylation of the 20S complex and myristoylation of the Rpt2 subunit activate the 26S proteasome whereas N-terminal acetylation has little effect on proteasomal activity *in vivo* (62).

N-terminal acetylation is a very common post-translational modification of eukaryotic proteins. About 50-80% of all eukaryotic proteins are acetylated in their N-termini. Several subunits of the 20S complex were shown to undergo N-terminal acetylation (see Table 1). It is hypothesized that N-acetylation of α-type subunits of the proteasome is important for the efficient substrate entering into the 20S proteolytic core complex in the absence of the19S regulatory subunit (61).

3.3. Role of proteasomes in cellular response to different forms of stress

Much experimental evidence supports the notion that proteasomes participate in adaptation of cells to different forms of stress (65). For example, the ATPase components of the 19S proteasome appear to be essential for the stress response in yeast. Chromatin immunoprecipitation has revealed that Sug1, Sug2 and Cim5/Rpt1/PSMC2 (another 19S ATPase subunit), but not the 20S proteasome core proteins, are recruited to promoters of stress-induced genes *HSP26*, *HSP104* and *GAD1* (66).

Likewise, the specificity and activity of the 26S proteasome alters in response to genotoxic stress, caused by topoisomerase II inhibitor, adriamycin (67). Combinatorial treatment of tumor cells with proteasome inhibitors and DNA damaging drugs was shown to enhance apoptosis (68, 69). Although the precise role of the proteasome in DNA damage-induced apoptosis is not yet clear, this effect may be mediated by inhibition of the proteasome-dependent degradation of the pro-apoptotic proteins Bax and Bid (70, 71).

Alternatively, inhibition of the proteasome may result in inactivation of membrane-bound P-glycoprotein, which confers multiple drug resistance (MDR) by enhancing efflux of drugs. Consequently, if a genotoxic drug is administered, its intracellular concentration and hence cytotoxicity will increase due to inability of P-glycoprotein to efficiently effuse the drug from the cell (72).

The fact that DNA damage regulates CKII and PKA kinases, which, in turn, are involved in regulation of the 26S proteasome indicates that proteasomes may confer cellular radio-resistance by a direct transcriptional activation of anti-apoptotic and/or DNA repair genes.

According to this hypothesis, DNA damage, by promoting association of CKII with the FACT complex and thus altering the specificity of the former, may decrease phosphorylation levels of the C8 (α7) subunit of the catalytic 20S complex (Figure 1, left) (73). This event may subsequently lead to dissociation of the 26S proteasome into 20S and 19S sub-complexes, thus allowing the 19S complex to employ its ATPase activity in transcriptional regulation (Figure 1, right). In addition, PKA-mediated phosphorylation of the Rpt6 subunit may further enhance ATPase activity of the 19S complex (74). Supporting this hypothesis is the fact that the 20S complex is more resistant to oxidative stress than the fully assembled 26S proteasome (75).

In line with the above model is a recently published study showing that in response to DNA damage, the proteasome activates the Fanconi anemia pathway, which, in turn, up-regulates cellular resistance to DNA cross-linking agents (76).

4. PERSPECTIVE

Aberrant activity of the 26S proteasome affects the cell cycle, apoptosis and other cellular processes related

to cancer (77). On the other hand, it is well established that the combinatorial treatment of cancer cells with radiomimetic drugs and inhibitors of proteasomes enhances apoptosis. However, as stated above, the mechanism by which proteasomes regulate apoptosis is yet not fully defined. It is also important to note that continuous treatment of cancer cells with proteasome-specific inhibitors decreases apoptosis and promotes assembly of inhibitor-resistant proteasomes (78, 79). Should our model is proved correct and DNA damage indeed enhances the dissociation of the proteasome by altering the specificity of CKII, then, by manipulating the level of CKII, it might be possible to increase the stability of the 26S proteasome and therefore the efficacy of combinatorial chemotherapy.

In sum, the aspect of proteasome-dependent regulation of transcription poses many important questions to be addressed. Deciphering how the proteasome utilizes its proteolytic and non-proteolytic activities to regulate transcription in response to various stresses will be instrumental to our understanding of the process of gene expression and tumorogenesis.

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