Review

Transcriptional regulation of the 26S proteasome by Nrf1

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Abstract: The 26S proteasome is a large protease complex that selectively degrades ubiquitinated proteins. It comprises 33 distinct subunits, each of which differ in function and structure, and which cannot be substituted by the other subunits. Owing to its complicated structure, the biogenesis of the 26S proteasome is elaborately regulated at the transcription, translation, and molecular assembly levels. Recent studies revealed that Nrf1 (NFE2L1) is a transcription factor that upregulates the expression of all the proteasome subunit genes in a concerted manner, especially during proteasome impairment in mammalian cells. In this review, we summarize current knowledge regarding the transcriptional regulation of the proteasome and recent findings concerning the regulation of Nrf1 transcription activity.

Keywords: proteasome, Nrf1, transcription, DDI2

Introduction

The ubiquitin proteasome system (UPS) is the main machinery for protein degradation in all eukaryotes. Substrate proteins are covalently modified by the addition of a small protein called ubiquitin, which is activated by the coordinated actions of ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3).1) The 26S proteasome (proteasome) is a huge protease complex that selectively recognizes and degrades ubiquitinated proteins in an ATP-dependent manner.2) Because the proteasome catalyzes the degradation of most of the proteins in cells, it plays an indispensable role in various biological processes such as DNA repair, cell cycle, immune responses, signal transduction, and protein quality control. Therefore, the dysregulation of proteasome function is associated with many human diseases. A decrease in proteasome activity is observed in autoinflammatory syndromes and neurodegenerative diseases including Alzheimer’s disease (AD), Parkinson’s disease (PD), and amyotrophic lateral sclerosis (ALS), whereas an increase in proteasome activity is observed in cancer cells, underscoring the importance of proper functional maintenance of the proteasome.3)–5) Because the proteasome comprises 33 distinct subunits, the biogenesis of the proteasome is elaborately regulated at several steps such as transcription, protein assembly, and post-translational modifications. In this review, we focus on the transcriptional regulation of the proteasome, especially in relation to recent findings in this subject.

Architecture of the proteasome

The 26S proteasome is composed of a 20S core particle (CP) and 19S regulatory particle (RP) that binds to either or both the ends of CP (Fig. 1).2,6) In the CP, the α-ring and β-ring individually comprise 7 distinct subunits, α1–α7 and β1–β7, respectively. These rings are stacked in an α-β-β-α topology to form a cylindrical shape complex (Fig. 1). The CP includes three proteolytically active subunits called β1, β2, and β5 in the β-ring and the active sites of these subunits are located inside the aforementioned cylindrical chamber. The central channel of the α-ring is usually closed and does not allow natively folded proteins to enter the proteolytic chamber.2) Recognition, unfolding, and translocation of substrate proteins towards the CP is performed by the RP. The RP is divided into two subcomplexes, namely, the base and lid. The base subcomplex contains six homologous AAA⁺ ATPase subunits (Rpt1–Rpt6) and four non-ATPase subunits (Rpn1,
Rpn2, Rpn10, and Rpn13) (Fig. 1). The six AAA⁺ ATPases form a ring and participate in the unfolding and translocation of substrates towards the interior cavity of CP in an ATP-dependent manner. Rpn10 and Rpn13 function as ubiquitin receptors.⁶,⁷ Rpn1 also exhibits binding capacity towards ubiquitin. Additionally, Rpn1 interacts with the ubiquitin-like domain, enabling the recruitment of shuttle factors such as human DNA repair protein (hHR23) and deubiquitinating enzyme (ubiquitin-specific protease 14; Usp14).⁸ Rpn2 interacts with Rpn13 that recruits another deubiquitinating enzyme (ubiquitin carboxy-terminal hydrolase, Uch37).⁹ Therefore, Rpn1 and Rpn2 serve as scaffolds to regulate efficient protein degradation. The lid subcomplex contains nine non-ATPase subunits, namely, Rpn3, Rpn5–Rpn9, Rpn11, Rpn12, and Rpn15/Dss1/Sem1 (Fig. 1). In the lid subcomplex, six subunits i.e., Rpn3, Rpn5–Rpn7, Rpn9, and Rpn12 exhibit a PCI (proteasome, COP9 signalosome, initiation factor 3) motif. These subunits mutually interact via their PCI motifs and form a horseshoe-like structure.¹¹ Rpn5 and Rpn6 interact directly with an α-ring subpart to stabilize the CP-RP interaction.¹² Rpn8 and Rpn11 are characterized by a MPN (Mpr1/Pad1 N-terminal) domain that is a putative metalloprotease catalytic domain. Rpn11 is a deubiquitinating enzyme that removes ubiquitin chains from the substrates, whereas Rpn8 lacks catalytic activity.¹³ Recently, Rpn15 was reported as a ubiquitin receptor. However, it is controversial whether Rpn15 can recruit ubiquitinated proteins to the proteasome, because its ubiquitin-binding surface overlaps with the proteasome-binding surface.⁹,¹⁴ Because the proteasome is a huge and complicated structure, proteasome assembly is regulated in a sophisticated manner.¹⁵ The CP and RP are constructed separately with the assistance of specific assembly chaperones. In CP assembly, PAC1–PAC4 mediate α-ring formation, and then Ump1/POMP orchestrates β-ring formation on the α-ring and the dimerization of half-CPs to form mature CPs. In
RP assembly, the base and lid subcomplexes are assembled independently. The base subcomplex assembly is mediated by four assembly chaperones, p28/gankyrin, p27, S5b, and Rpn14/PAAF. In contrast, there appear to be no specific assembly chaperones to assemble the lid subcomplex, because it can be reconstituted by the exogenous expression of lid subunits in E. coli.20)

Transcriptional regulation of proteasome genes in yeast and mammals

Each proteasome subunit exhibits a distinct structure and specific function that cannot be substituted by the other subunits; therefore, the expression of all the proteasome subunits is regulated coordinately at the transcription level. This regulatory system was initially identified in Saccharomyces cerevisiae (budding yeast). In yeast, the coordinated expression of proteasome genes is mediated by the transcription factor Rpn4, which binds to a specific sequence motif, proteasome-associated control element (PACE), present in the promoter regions of all proteasome subunit genes and a few proteasome assembly chaperone genes. Therefore, Rpn4 is required to maintain the constitutive expression of proteasome subunits.16),17) Intriguingly, Rpn4 has an extremely short half-life (t_{1/2} \approx 2 \text{ min}), because it is continually degraded by the proteasome.18) Therefore, when proteasome function is compromised, Rpn4 accumulates and augments proteasome expression to recover proteasome function. This compensatory response (called bounce-back response) is indispensable to maintain adequate proteasome activity. In fact, the deletion of either Rpn4 or a PACE sequence in one of the proteasome subunit genes remarkably decreases proteasome activity and sensitizes cells to stresses, including DNA damage and oxidation.19)

A similar regulatory mechanism exists in mammalian cells. It was reported that the impairment of proteasome activity promotes the concerted expression of all proteasome subunit genes and enhances compensative de novo proteasome synthesis in mammalian cells.20) However, sequence homology searches were unable to identify a mammalian ortholog of Rpn4. Moreover, PACE sequences were absent in the promoter regions of mammalian proteasome subunit genes, suggesting that the molecular mechanism of proteasome gene expression in mammals differs from that in yeast.20) Instead, the transcription factors nuclear transcription factor Y (NF-Y), forkhead box protein O4 (FOXO4), and signal transducer and activator of transcription 3 (STAT3) were identified as regulators of constitutive proteasome expression.21)–23) NF-Y regulates the expression of six CP subunit genes (α2, α5, α7, β3, β4, and β6), five RP subunit genes (Rpt1, Rpt5, Rpt6, Rpn10, and Rpn11), and one assembly chaperone (p28), which contain one or more CCAAT box in their promoter region.21) FOXO4 promotes Rpn6 expression, which contributes to high proteasome activity in pluripotent stem cells.22) STAT3 regulates the expression of β-subunit genes and mediates epidermal growth factor (EGF)-induced proteasome upregulation in several cancer cells.23) These reports suggested that multiple transcription factors maintain constitutive proteasome expression, which depends on the promoter sequence of each gene, cell types, and cellular conditions in mammalian cells. However, the mechanism by which the expression of a particular set of subunits but not all the subunits increases the quantity of completely assembled proteasomes remains unexplained. Recently, it was demonstrated that the bounce-back response in mammals is caused by the transcription factor nuclear factor erythroid-derived 2-related factor 1 (Nrf1). Nrf1 knockdown or knockout suppresses the upregulation of proteasome gene expression and de novo proteasome synthesis in response to proteasome inhibition. This indicates that Nrf1 plays a pivotal role in the regulation of mammalian proteasome concentration subsequent to proteasome dysfunction.24),25)

Molecular characteristics of the transcription factor Nrf1

Nrf1 is a cap ‘n’ collar basic leucine zipper (CNC-bZIP) family transcription factor. In mammals, this family includes six members, Nrf1,26) Nrf2,27) Nrf3,28) nuclear factor erythroid 2 (NF-E2) p45 subunit,29),30) BTB and CNC homolog 1 (Bach1),31) and Bach2.31) All these proteins contain a CNC domain and bZIP domain (Fig. 2).

The bZIP domain located near the C-terminus of Nrf1 is characterized by a 30-amino acid region enriched in arginine and lysine residues and a 40-amino acid helical region containing heptad repeats of leucine and hydrophobic residues.26),32) The former region is responsible for DNA binding, whereas the latter region is necessary for dimerization with other bZIP proteins called small musculoaponeurotic fibrosarcoma (Maf), including MafF, MafG, and MafK. Additionally, because small Maf proteins bind to DNA through its bZIP domain, heterodimerization with a small Maf significantly promotes the DNA
binding efficiency of a CNC-bZIP protein. The CNC domain is named after the Drosophila cap and collar gene and is a 43-amino acid region that flanks the N-terminal end of the bZIP domain. Its sequence is conserved in homologous transcription factors in insects, worms, fish, birds, and mammals, but not in plants and fungi. This domain is necessary for the DNA binding and transactivation capacity of any CNC-bZIP protein; however, the precise mechanism is unknown.

A heterodimer of a CNC-bZIP protein and a small Maf protein binds to the antioxidant response element (ARE). AREs comprises a 5'-RTGACnnnGC-3' (R = A or G) core sequence and are located in the enhancer and promoter regions of various genes mainly involved in the antioxidant response and metabolic regulation. Recently, chromatin immunoprecipitation (ChIP)-seq data identified 5'-RTGACTCAGC-3' as the consensus binding site of Nrf1. Notably, this binding site exists in the enhancer or promoter region of all the 33 proteasome subunit genes. Therefore, Nrf1 coordinately activates proteasome gene expression. In fact, the expression of proteasome subunits is dependent on Nrf1, but not Nrf2, subsequent to proteasome inhibition. However, in a few kinds of cancer cells, Nrf2 enhances proteasome expression. Nrf2 selectively interacts with p53 gain-of-function mutants that increase the binding capacity of Nrf2 to proteasome gene promoters. This suggests that the association with other factors plays an important role in determining the binding specificity of Nrf1 and Nrf2, but the precise mechanisms remain to be elucidated.

Nrf1 is a short-lived protein that is degraded by the UPS

Similar to yeast Rpn4, Nrf1 is degraded by the UPS (Fig. 3). Newly synthesized Nrf1 enters the endoplasmic reticulum (ER) and gets embedded into the ER membrane via a transmembrane domain at its N-terminus. Generally, ER-resident proteins are degraded by the ER-associated protein degradation (ERAD) pathway when they are misfolded. Notably, Nrf1 is constantly subjected to ERAD. In ERAD, ER proteins are ubiquitinated by ER-resident ubiquitin ligases, extracted from the ER into the cytosol by AAA+ ATPase p97/valosin containing protein (VCP), and degraded by the proteasome. In fact, ubiquitination by the ER-resident ubiquitin ligase Hrd1 and the ATPase activity of p97 are required for Nrf1 degradation, because the depletion of either Hrd1 or p97 prevented

Fig. 2. Domain organization of CNC-bZIP family proteins. The image depicts the domain organization of six human CNC-bZIP proteins, namely, Nrf1, Nrf2, Nrf3, NF-E2 (p45 subunit), Bach1, and Bach2. All these proteins share cap ‘n’ collar (CNC) and a basic leucine zipper (bZIP) domains in their C-terminus region. Nrf1, Nrf2, Nrf3, and NF-E2 p45 contain one or two acidic domains (AD1, AD2, or AD2L) and regulate the transcriptional activation. In contrast, Bach1 and Bach2 are known as transcription repressors without acidic domains. However, they contain a broad-complex, tramtrack, bric-a-brac (BTB) domain. Nrf1 and Nrf3 exhibit a N-terminal domain (NTD) with a transmembrane region to anchor proteins to the ER membrane. Additionally, Nrf1 and Nrf3 contain an Asn/Ser/Thr-rich (NST) domain, which is a target for N-glycosylation.
the ERAD of Nrf1. Moreover, Nrf1 stability is regulated in the nucleus. Beta-transducin repeat containing protein (β-TrCP), which forms a Skp1-Cul1-Fbox (SCF) ubiquitin ligase complex recognizes the DSGLS sequence in Nrf1 and ubiquitinates Nrf1 for degradation. Additionally, nuclear Nrf1 is ubiquitinated by another SCF family ubiquitin ligase FBXW7 and degraded by the proteasome. These ubiquitin ligases contribute to the short half-life of Nrf1, i.e., <30 min. Therefore, Nrf1 is maintained at a low level in normal conditions, whereas Nrf1 accumulates to high levels when proteasome activity is compromised and exerts a compensative effect on proteasome expression.

**Retrotranslocation from the ER and proteolytic processing are required for Nrf1 activation**

Recent studies clarified that Nrf1 is retrotranslocated from the ER, deglycosylated, and proteolytically cleaved to produce the active form that translocates to the nucleus in proteasome-inhibited conditions. The nascent Nrf1 is tethered to the ER membrane via its N-terminal transmembrane domain, and a large proportion of its polypeptide is in the ER lumen and is N-glycosylated (Fig. 4). Therefore, the abrogation of p97/VCP causes the accumulation of glycosylated Nrf1 and completely blocks the bounce-back response after proteasome inhibition.

In cells treated with a proteasome inhibitor, Nrf1 escapes degradation by the proteasome and is accumulated as two main forms, both of which do not correspond to glycosylated Nrf1. The high molecular weight form (~120 kDa) is anchored to the ER membrane, whereas the low molecular weight form (~110 kDa) localizes in the nucleus and exhibits transcription activity (Fig. 4). For several years, it remained unclear whether the mechanism generat-
ing these species involved processing, glycosylation, or phosphorylation.\(^{42,43}\) A recent study clarified that the \(\sim110\) kDa species is generated by processing. Edman-degradation assays confirmed that the N-terminus of the \(\sim110\) kDa form starts with the Leu104 residue.\(^{46}\) The processed \(\sim110\) kDa form of Nrf1 lacks the N-terminal transmembrane domain compared with the \(\sim120\) kDa full-length form of Nrf1, supporting the view that it is liberated from the ER membrane. Moreover, amino acid substitutions surrounding the processed site blocked the processing, suggesting that a specific protease yields the processed form of Nrf1.

The activation mechanisms of other ER-resident transcription factors such as sterol regulatory element-binding protein (SREBP), activating transcription factor 6 (ATF6), OASIS, and cyclic AMP-responsive element-binding protein H (CREBH) have been closely studied to date. All these proteins transit to the Golgi and are cleaved by site-1 protease (S1P) and site-2 protease (S2P) and released from the membrane.\(^{48-52}\) However, Nrf1 processing occurs in the absence of S1P, S2P, ER membrane-resident rhomboid proteases, or the proteasome.\(^{43,46}\) Additionally, the transcription activation domain and DNA binding domain of Nrf1 reside inside the ER lumen, whereas those of the other ER-resident transcription factors are present inside the cytosol. Therefore, the Nrf1 activation mechanism was considered to be a non-canonical pathway and its precise mechanism remained unknown.

**DNA-damage inducible 1 homolog 2 (DDI2)**

Recently, a genome-wide siRNA screening using human cell cultures was performed to achieve a comprehensive understanding of the Nrf1 activation mechanism. Because Nrf1 translocation from the ER
to the nucleus coincides with Nrf1 activation, the genes responsible for nuclear translocation of Nrf1 were screened using image-based analysis. As a consequence, a putative aspartic protease, DDI2, was identified as a Nrf1 nuclear translocation regulator.53) DDI2 is an ortholog of Saccharomyces cerevisiae DNA-damage inducible 1 (Ddi1, also known as Vsm1). Ddi1 participates in the DNA-damage checkpoint54),55) and protein secretion,56)–58) whereas very little is known regarding the physiological roles of DDI proteins in mammals. However, DDI family proteins contain a retroviral protease-like (RVP) domain that exhibits a structure that closely resembles that of HIV protease-1, suggesting that DDI proteins are aspartic proteases.59)–61) In fact, a mutation in the protease active site of DDI2 decreased the production of the ~110 kDa processed form of Nrf1, whereas the ~120 kDa full-length form of Nrf1 accumulated. Additionally, the bounce-back response of proteasome expression against proteasome inhibition was remarkably suppressed by DDI2 depletion and a protease-dead DDI2 mutant.54)

Moreover, the same phenomenon was observed by performing genetic screening using Caenorhabditis elegans (a nematode). In this screening, skinhead 1 (skn1; an ortholog of Nrf1)-dependent induction of proteasome gene expression was monitored using rpt3 promoter-driven GFP expression. During the search for suppressors of GFP expression, mutants of ddi1 (a C. elegans ortholog of DDI2) were identified. A defect in ddi1 activity suppressed skn1 processing. This indicated that the activation mechanism of Nrf1/Skn1 is widely conserved in multicellular animals.62)

Functions of the atypical ubiquitin-like domain in DDI2

Yeast Ddi1 contains ubiquitin-like (UBL) and ubiquitin-associated (UBA) domains at the N-terminus and C-terminus, respectively. Other UBL-UBA containing proteins such as Rad23 and Dsk2 are shuttle factors that interact with ubiquitinated substrates and proteasome subunits through their UBA and UBL domains, respectively, for protein degradation via the UPS. However, yeast Ddi1 exhibited weaker binding affinity toward ubiquitin chains than Rad23 and Dsk2, despite a similar domain composition.63) Although yeast Ddi1 was reported to be involved in the degradation of a few proteins, its function as a shuttle factor remains unestablished.55),64)

The mammalian orthologs of yeast Ddi1, including human DDI2, lack the C-terminal UBA domain. However, they contain a ubiquitin-interacting motif that weakly binds to ubiquitin.61) Furthermore, the UBL domain of Ddi family proteins exhibits an unconventional feature compared with ubiquitin or other UBL proteins. Generally, the hydrophobic patch, which is the interaction site of binding partners, is surrounded by positively charged side chains in ubiquitin and ordinary UBL domains. However, the hydrophobic patch of the Ddi UBL domain is surrounded by negatively charged side chains, suggesting that the binding partners of the Ddi UBL domain are distinct from those of ubiquitin and other UBL domains.65) In fact, it was demonstrated that Ddi UBL is an atypical UBL domain that binds to ubiquitin.64)

The UBL domain of human DDI2 is involved in Nrf1 processing,53) probably through interaction with ubiquitin chains. Consistent with this, blocking ubiquitin chain formation using an E1 inhibitor causes the accumulation of full-length Nrf1 (~120 kDa) despite the presence of DDI2.47) Further analysis is required to reveal the mechanism by which DDI2 recognizes the substrate and becomes activated. These studies contributed to clarify the detailed molecular mechanisms of proteasome upregulation.

Posttranslational modification of Nrf1

In addition to proteolytic processing, Nrf1 transcription activity is regulated by phosphorylation and glycosylation (Fig. 5). Phosphorylation is involved in positive as well as negative regulation of Nrf1 transcription activity. Treatment with a phosphatase inhibitor okadaic acid increased Nrf1 transactivation activity, whereas a protein kinase C (PKC) inhibitor staurosporine suppressed this activity in an ARE-driven luciferase assay, suggesting the positive effect of phosphorylation on Nrf1 activity.66) However, there is no evidence that indicates the direct phosphorylation of Nrf1 by PKC.

In contrast, casein kinase 2 (CK2) and glycogen synthase kinase 3 (GSK3β) directly phosphorylate Nrf1 to suppress its transcription activity. CK2 interacts with Nrf1 and phosphorylates the Ser497 residue. CK2 knockdown enhances the recruitment of Nrf1 to the AREs of promoter regions of proteasome subunit genes to upregulate their expression levels.67) GSK3β binds to Nrf1 and phosphorylates the Ser350 residue. GSK3β inhibitor treatment or the GSK3β S350A mutant stabilizes Nrf1, because the phosphorylation of Ser350 by GSK3β is required for
F-box/WD repeat-containing protein 7 (FBW7)-dependent Nrf1 degradation. Additionally, the expression of Nrf1 S350A mutant ameliorated stress-induced apoptosis in neuronal cells, indicating the importance of the GSK3β-mediated regulation of Nrf1 activity.68)

As discussed previously, Nrf1 is modified by N-linked glycosylation in the ER lumen. The glycosylation occurs in its Asn/Ser/Thr-rich (NST) domain, which consists of seven Asn-X-Ser/Thr consensus motifs for glycosylation. Recent reports indicated that glycosylation regulates Nrf1 transcription activity. Glycosylation-defective Nrf1, in which all the Asn residues are replaced with Gln residues exhibits a decreased transcription activity, whereas an Nrf1 mutant that mimics the deglycosylated state by the replacement of all the Asn residues with Asp residues exhibits enhanced transcription activity.69) Furthermore, genetic screening in nematode demonstrated the function of N-glycanase 1 (png-1), which is an ortholog of human N-glycanase 1 (NGLY1) responsible for the bounce-back response of proteasome gene expression.62) Accordingly, human NGLY1 is required for Nrf1 transcription activity because it promotes the Nrf1 processing and nuclear translocation.70) These data indicated that N-linked glycosylation and subsequent deglycosylation steps exhibit critical roles in Nrf1 function.

In addition to N-linked glycosylation, O-linked glycosylation is believed to be involved in Nrf1 transcription activity. The previous genome-wide screening in mammalian cells identified O-linked N-acetylglucosamine transferase (OGT) as a positive regulator of Nrf1 transcription activity.53) This finding was supported by a recent report that OGT increases Nrf1 stability and transcription activity.71) OGT interacts with Nrf1 via host cell factor 1 (HCF1) and modifies Nrf1 with O-linked N-acetylglucosamine, which attenuates the ubiquitination of Nrf1 prior to degradation.72) In contrast, OGT is also known to be involved in the negative regulation of Nrf1 activity. In Nrf1, the serine/threonine-rich region in the proline-glutamate-serine-threonine-rich sequence 2 (PEST2) is involved in O-linked glycosylation that down-regulates Nrf1 stability and transcription activity.73) The dual role of O-linked glycosylation might regulate Nrf1 activity in response to various cellular conditions.

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**Fig. 5.** Regulation of Nrf1 transcription activity by phosphorylation and glycosylation. Nrf1 is phosphorylated by CK2 and GSK3β which decreases the transcription activity. PKC kinase activity is reported to enhance Nrf1 transcription activity; however, whether PKC phosphorylates Nrf1 directly remains unclear. Additionally, glycosylation status affects Nrf1 transcription activity. Nrf1 is N-glycosylated in the ER and deglycosylated by NGLY1 in the cytosol. The deglycosylation promotes Nrf1 transcription activity and processing by DDI2. The O-glycosylation of Nrf1 by OGT positively as well as negatively regulates Nrf1 activity.
Nrf1 regulation machinery as a potential therapeutic target

Multiple lines of evidence have revealed the association between proteasome function and various diseases such as cancers, neurodegeneration, autoinflammation, and autoimmunity. Presently, the inhibition of proteasome activity was demonstrated to exhibit prominent effects in the treatment of multiple myeloma and other malignant neoplasms. However, the emergence of drug resistance against proteasome inhibitors is a major concern during cancer treatment. The bounce-back response mediated by Nrf1 activation is one of the causes of drug resistance. In fact, increased expression of the β5 subunit, which is a target subunit of proteasome inhibitor bortezomib, contributes to drug resistance in lymphoma cells. In contrast, Nrf1 knockdown augments sensitivity to proteasome inhibitors in tumor cell lines. Therefore, it might be promising to use a combination of proteasome inhibitor and Nrf1 suppressor to improve cancer therapy.

In this regard, Nrf1 and molecules involved in processing, glycosylation, phosphorylation, and degradation of Nrf1 are promising pharmacological targets. Indeed, Nrf1, DDI2, and NGLY1 exhibit a correlation in being essential for cell survival in several acute myeloid leukemia (AML) cell lines. Knockdown of DDI2 and NGLY1 potentiated sensitivity against a proteasome inhibitor. Additionally, an NGLY1 inhibitor synergistically promoted cytotoxicity in presence of a proteasome inhibitor. These results support the perspective of drug discovery to target Nrf1 activation mechanisms.

Concluding remarks

Proteasome function is regulated by various molecules in response to multiple cellular events. Many studies have focused on the complicated molecular systems that regulate proteasome activity; however, complete information regarding these systems remains unknown. Among these pathways, Nrf1 plays an indispensable role in de novo proteasome synthesis in response to the compromised proteasome activity. Recent studies have unveiled the regulatory mechanisms of Nrf1 activation; however, many questions remain unanswered. Whereas most of the ER-resident transcription factors exhibit a membrane topology that localizes the domains required for their transcription activity into the cytosol, the major part of the polypeptide chain of Nrf1 is located in the ER lumen. However, the significance of membrane topology that retains Nrf1 in the ER is unknown. Additionally, though DDI2 is the processing enzyme for Nrf1 activation, the mechanism of DDI2 activation is unclear. Because the protease activity of purified recombinant DDI2 is not confirmed in vitro, some co-factors or modifications might be needed for DDI2 activation.

Proteasome activity is associated with many human diseases and in cell physiology. A decrease in proteasome activity is associated with senescence, neurodegeneration, and autoinflammation, whereas maintenance of a high proteasome activity plays an essential role in the pluripotency of embryonic stem cells as well as survival of malignant cells. Therefore, further studies regarding the regulatory mechanisms of proteasome expression are essential to provide new strategies to improve healthy aging and treat various diseases.

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Profile

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