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Regulation of gene expression by the ubiquitin-proteasome system

Tingting Yao and Ada Ndoja

Colorado State University, Biochemistry and Molecular Biology, 1870 Campus Delivery Fort Collins, CO 80523, United States

Tingting Yao: Tingting.Yao@Colostate.edu

Abstract

Transcription is the foremost regulatory point during the process of producing a functional protein. Not only specific genes need to be turned on and off according to growth and environmental conditions, the amounts and quality of transcripts produced are fine-tuned to offer optimal responses. As a result, numerous regulatory mechanisms converge to provide temporal and spatial specificity for this process. In the past decade, the ubiquitin-proteasome system (UPS), which is best known as a pathway for intracellular proteolysis, has emerged as another pivotal player in the control of gene expression. There is increasing evidence that the UPS has both proteolytic and non-proteolytic functions in multiple aspects of the transcription process, including initiation, elongation, mRNA processing as well as chromatin dynamics. In this review, we introduce the many interfaces between the UPS and transcription with focuses on the mechanistic understanding of UPS function in each process.

Keywords

ubiquitin; transcription; proteasome; activator; RNA polymerase II

1. Introduction

1.1 The diverse functions of ubiquitin modification

In eukaryotes, the covalent attachment of the small protein ubiquitin (Ub) to other proteins regulates multiple and diverse aspects of cell physiology, including endocytosis, cell cycle, DNA repair, transcriptional regulation, and chromatin dynamics [1]. In many respects, ubiquitylation is analogous to phosphorylation and its function is analogous to signaling by protein kinases. However, the Ub signal is structurally more versatile (Fig. 1A). Because Ub can be attached to other Ub molecules to form polyUb chains, the type of Ub modification can differ in the number of Ub molecules attached to the substrate and the linkage between the Ubs in a polyUb chain [2–4]. Ub has seven lysines and an N-terminal amine, each of which can engage in polyUb chain formation *in vivo* [5]. Classically, modification by a K48-linked polyUb chain leads to targeting to the 26S proteasome and subsequent degradation of the substrate. More recent studies have shown that the functions of mono-ubiquitylation and other types of polyUb linkages are much more diverse [6–8]. In

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particular, mono-ubiquitylation of transcription activators and histone proteins do not lead to protein degradation [9–11]. In this review, we will mainly discuss the proteolytic functions of Ub modification in controlling gene expression, but also some of its non proteolytic functions.

1.2 The proteasome assemblies

The proteasome is the central machinery that handles regulated proteolysis in cells. The 20S core proteasome (CP) is a cylinder-shaped particle composed of four 7-subunit rings (Fig. 1B) [12]. The rings are stacked to form a self-compartmentalized protease complex with a C2 symmetry. The two inner rings contain seven distinct β -type subunits, of which three have proteolytic activities, whereas the two outer rings contain α -type subunits that control access to the proteolytic sites sequestered within the cylinder's interior. By itself, the 20S CP can only degrade unfolded polypeptides. It is the 19S Regulatory Particle (RP) that ties the 20S CP to the Ub system. Consisting of at least 18 different subunits, the 19S RP orchestrates all the steps that lead to degradation of ubiquitylated proteins [13]; these include recognition of (poly)Ub, substrate unfolding, substrate deubiquitylation, and substrate translocation into the 20S catalytic core [14]. The 26S proteasome is formed upon binding of a 19S RP to one or both ends of the 20S CP.

Although the distribution and dynamics of 19S, 20S, and 26S particles in cells are not well understood and may vary with cell type and growth conditions, it is generally believed that the 19S RP exists both as an independent complex and as part of the 26S proteasome [15]. By itself, the 19S RP has non-proteolytic functions through interactions with ubiquitylated proteins [16, 17]. There is also evidence that it directly interacts with some transcription activators and co-activators [18–21]. The 19S RP can be further divided into two subassemblies: the Base and the Lid [22]. The Base, also known as APIS (ATPase Independent of 20S), is comprised of six ATPases (Rpt1–6), Rpn1, Rpn2, and Rpn13. It contains (poly)Ub recognition and protein unfolding activities and has been implicated in transcription activation [16, 19, 23–25]. However, it has not been demonstrated that the Base exists as an independent complex *in vivo*.

2. Regulation of transcription activators by the ubiquitin-proteasome system (UPS)

Transcription activators have an important role in coordinating the assembly of complex protein machineries – RNA polymerase II (RNAPII), general transcription factors, and various co-activators with chromatin modifying and remodeling activities – with the goal to activate gene expression in a time and locus specific manner. Research from multiple laboratories has revealed that the UPS functions in the regulation of transcription activators by limiting their abundance and also by assisting productive transcription in a variety of ways.

2.1 Coupled transcription activation and UPS-dependent degradation of the activator protein

At first glance, it seems that the classical Ub-proteasome pathway is perfectly suited for regulating the amounts of specific transcription activators in the cell. For a subset of activators that include HIF-1 α , inhibition of their ubiquitylation prevents them from being degraded by the proteasome; this leads to accumulation of the activator and increased transcription of its target gene(s) [26]. However, for another subset of activators, their turnover is coupled to their transcriptional activities. In 1999 Molinari *et al.* reported that activators with high transcription potency are often short-lived proteins [27]. Subsequent

work from multiple laboratories showed that there is an interdependence between transcription activation and degradation of the activator protein [28].

First, there is an inverse relationship between transcription potency and activator half-life. Salghetti *et al.* constructed a series of synthetic activators consisting of the yeast Gal4 DNA binding domain (DBD) fused to tandem copies of a short acidic fragment (VN8) derived from Herpes Simplex virus protein VP16 [29]. With more copies of VN8, higher transcription was observed together with shorter half-lives of the activator. Conversely, mutations in VN8 that attenuated its transcription potency increased its half-life. Second, inhibition of UPS-mediated degradation not only can stabilize the activator but also inhibited transcription activation. In budding yeast, transcription activation by Gcn4 and Gal4 are inhibited by proteasome inhibitor MG132 [30, 31]. In mammalian cells, Myc and estrogen receptor alpha (ER α) are two of the best-studied cases where transcription activation is inhibited either by proteasome inhibition or by inactivation of the ubiquitylation machinery [32–34]. More recently, Wang *et al.* constructed a series of non-classical synthetic activators consisting of the bacterial LexA DBD fused to the C-terminus of each of 17 Mediator subunits [35]. Mediator is a large protein complex that interacts with RNAPII and plays essential roles in activator-dependent transcription. A Med-LexA fusion is predicted to recruit the Mediator to LexA-binding sites and activate transcription from a reporter gene. Among the 17 subunits only 3 that reside in the tail module of the Mediator activated transcription when fused to LexA, which may be a result of structural constraints that are imposed by the artificial fusion. However, all 3 fusions that are capable of activating transcription were short-lived proteins. Proteasome mutations that stabilize these fusions also inhibited transcription of the reporter gene. This observation lends further support to the hypothesis that activator degradation is an intrinsic requirement for certain types of transcription, such as those that require direct interactions between the activator and the Mediator.

Interestingly, for many natural activators, their transcription activation domains (ADs) overlap with the sequences that are responsible for Ub-dependent degradation (degrons). Examples include ATF6, E2F-1, ER α , Fos, Gcn4, IRF-1, Jun, Myc, and p53 [28, 29]. The co-localization of ADs and degrons suggests coupling between transcription activation and degradation. However, it is generally unclear if degradation is merely a consequence of transcription or whether it also plays a positive role in transcription activation. Studies of ER α suggest that in this case activator degradation is required to promote multiple rounds of transcription. By following synchronized induction of estrogen-response genes, Metivier *et al.* showed that ER α and transcription machinery are recruited to a promoter in a cyclic manner, each cycle corresponding to one round of transcription [33, 36]. At a late point of each cycle, E3 Ub ligases and components of the proteasome are recruited, followed by disappearance of the activator from the promoter. Inhibition of the proteasome prolonged the presence of the activator at the promoter and prevented additional transcription cycles. These observations led to a model in which, after each round of transcription, the activator and other components of the transcription machinery must be cleared from the promoter through proteasome-dependent degradation of the activator (Fig. 2A).

Another study suggests that activator ubiquitylation and degradation may also play an important role in mRNA processing. The F-box is a protein motif that can associate with other proteins to form a SCF-type of E3 Ub ligase [37]. Yeast strains that lack the F-box protein Dsg1 (also known as Mdm30) cannot use galactose as carbon source. Muratani *et al.* found that Dsg1 is involved in ubiquitylation and degradation of a specific pool of Gal4 that is engaged in transcription [31]. These Gal4 molecules are marked by S699 and S837 phosphorylation, mediated by components of the transcription pre-initiation complex. Phosphorylated Gal4 is ubiquitylated by SCF^{Dsg1} and subsequently degraded by the

proteasome. In the absence of Dsg1, there is a defect in phosphorylation of RNAPII at Ser2 positions within the C-terminal Domain (CTD). This defect did not affect the amounts of the transcripts produced or mRNA export, yet these transcripts were poorly translated, indicating a defect in the quality of the transcripts. Although the detailed mechanisms remain unclear, it is possible that failure to degrade Gal4 leads to inefficient disassembly of the initiation complex, and/or recruitment of mRNA processing factors.

It is important to keep in mind that there may be more than one E3 Ub ligases for a given transcription activator, and each may play a different role in regulating activator function. In the case of Gal4, whereas Dsg1 regulates the pool engaged in transcription, another F-box protein, Grr1, regulates the overall abundance of the protein [31]. When yeast are grown in raffinose, Grr1 helps to keep the *GAL* genes repressed by limiting total amounts of Gal4 in the cell. Deletion of Grr1 leads to derepression. When the carbon source is switched to galactose, Dsg1-dependent degradation of Gal4 plays a positive role in transcription, opposite to Grr1. A similar case in mammals is seen with the oncoprotein Myc. Whereas the overall abundance of Myc is regulated by the F-box protein Fbw7 [38, 39], transcription activation by Myc requires a different F-box protein, Skp2 [32, 34]. As the ability to identify E3 ligase substrates improves, we are likely to see more cases like those of Gal4 and Myc. Transcription-coupled phosphorylation is likely a general strategy to mark the subset of an activator that is engaged in transcription [40]. By using multiple E3 Ub ligases that recognize different pools of the activator, the cell can fine-tune its response to changing environments. Thus, it may be no coincidence that activators known to be regulated by the UPS are involved in induced rather than constitutive transcription.

2.2 Non-proteolytic roles of UPS in transcription

Transcription-coupled degradation typically involves polyubiquitylation of the activator. Interestingly, there are a few cases where mono-ubiquitylation of the activator plays a non-proteolytic role in transcription [18, 21, 41–43]. In a pioneering study, Salghetti *et al.* demonstrated that a Ub-activator fusion protein can bypass the need for the ubiquitylation machinery and activate transcription directly [44]. They constructed a heterologous activator consisting of LexA DBD and VP16 AD. When introduced into yeast, LexA-VP16 is a potent activator with a half-life of ~3 minutes. Transcription activation and degradation of LexA-VP16 both depend on the yeast F-box protein Met30 – in the absence of Met30, LexA-VP16 no longer activates transcription. However, transcription is restored if monoUb is fused to the N-terminus of LexA-VP16. Surprisingly, the fused Ub does not lead to turnover of the activator, which suggests that the monoUb signal can activate transcription independent of proteolysis.

Another example comes from a series of studies from the Kodadek and Johnston laboratories on Gal4 [18, 41, 45–48]. Using an *in vitro* system containing HeLa nuclear extract, they found that Gal4-VP16 is rapidly displaced from DNA in an ATP-dependent manner by the 19S RP [18]. Interestingly, a small fraction of Gal4-VP16 is mono-ubiquitylated by the extract and able to bind to DNA stably. Similarly, a Ub-Gal4-VP16 genetic fusion also can stably bind to DNA (Fig. 2B). Such behavior is mirrored by a Gal4 mutant named Gap71, which contains three point mutations in the DBD of native Gal4. In the absence of nuclear extract, Gap71 has an affinity for DNA similar to native Gal4. However, *in vivo*, Gap71 does not activate *GAL* genes efficiently and is not detected at the promoters upon galactose induction. A mutation in the proteasome ATPase Rpt6 (*sug1–20*) can partially rescue the transcriptional defects of Gap71. Both genetic and biochemical evidence suggest that Gal4 directly interact with two ATPases of the 19S RP, Rpt4 and Rpt6 (also known as Sug2 and Sug1, respectively) [19, 41]. It was proposed that these interactions remove Gal4 from DNA through the unfoldase activities of the 19S RP. Mono-ubiquitylation of Gal4 may protect against this removal from the DNA, thereby promoting

transcription. The failure of the Gap71 mutant to activate transcription may be due to defective ubiquitylation. [18, 41, 45, 46, 49].

Although the generality of activator mono-ubiquitylation is unclear, the studies cited above demonstrate that the UPS can regulate activator proteins via multiple mechanisms. Because transcription is a complex process involving initiation, elongation, termination and reinitiation, it has been proposed that monoUb and polyUb signals are used at different stages of this process [11, 50]; although convincing evidence for this model is lacking. Added to this complexity are the observations that different proteasome assemblies may be recruited by different activators. By chromatin immunoprecipitation (ChIP), Gonzalez *et al.* demonstrated that the APIS subcomplex of the 19S RP is recruited to the yeast *GAL* genes upon induction with galactose [19]. The absence of 20S components suggested that the proteasome plays a non-proteolytic role in *GAL* activation. However, several limitations inherent to the ChIP technique warrant caution in this interpretation. Genome-wide association studies showed that both 19S and 20S components are widely distributed across the yeast genome, suggesting prevalent involvement of the proteasome in transcription regulation [51–53]. What their functions are at different gene loci are among the major questions for future studies in this field.

Besides activators, the 19S RP has been shown to interact with a transcription co-activator SAGA (Spt-Ada-Gcn5-Acetyltransferase) [20, 54]. SAGA, which plays a major role in induced transcription in yeast and higher eukaryotes, is a multi-functional complex consisting of several modules. The acetyltransferase module acetylates multiple lysines in histone H3 to promote transcription initiation, whereas the deubiquitylase module regulates transcription elongation and mRNA export (further discussed in Section 4). *In vitro*, the 19S RP stimulates recruitment of SAGA by Gal4-VP16 in an ATP-dependent manner [20]. *In vivo*, mutations that inactivate any 19S RP ATPase subunit prevent stable assembly of transcription pre-initiation complexes at the *GAL1* promoter [54]. It was proposed that interactions between the activator, 19S RP, and SAGA, although only weak as pairwise interactions, significantly stabilize the ternary complex (Fig. 2C). Consistently, a mutation in the ATPase domain of yeast Rpt6 (*sug1–25*) leads to reduced levels of H3 acetylation globally [20]. The 19S RP has also been shown to enhance elongation both *in vitro* and *in vivo* [55–57], but whether these effects are mediated through interactions with SAGA is unknown.

Finally the UPS also has been implicated in transcription repression through both proteolytic and non-proteolytic mechanisms. Met4 is a master regulator of sulfur metabolism in yeast [58]. When yeast is grown in minimal medium, Met4 activates transcription of a large number of genes involved in synthesis of sulfur-containing metabolites. Upon addition of methionine, Met4 is ubiquitylated by the SCF^{Met30} E3 ligase, which rapidly turns off gene expression. While Met4 polyubiquitylation can lead to its rapid degradation by the proteasome [59, 60], this is not necessarily the case. Indeed, Met4 modified by a canonical K48-linked Ub chain generally remains stable [61, 62]. Indeed, the Kaiser group identified two internal Ub-binding domains in Met4 that sequester the Ub chain *in cis* and prevent Met4 from being targeted to the proteasome [63]. Although the mechanism remains unclear, ubiquitylation of Met4 is thus sufficient to turn off gene expression without invoking proteolysis.

Discussion—Although many studies have revealed multiple modes of regulation of transcription by the UPS, each study typically focuses on only one mode and many controversies remain. Is there a unified mechanism that underlies the coupling of transcription and degradation of the activator? Why is degradation of the activator necessary in some cases and not in others? What are the different functions of chromatin-associated

19S or 20S proteasome complexes at different gene loci? How is recruitment of different proteasome assemblies controlled? Answers to these questions are needed to resolve controversies and to bring the big picture into focus.

3. Ubiquitylation of RNA Polymerase II

Either DNA damage or transcriptional arrest can induce ubiquitylation and degradation of the largest subunit of RNAPII, Rpb1 [64]. Because DNA damage likely induces DNA lesions that ultimately cause transcriptional arrest, here we will focus on the function, signals and enzymatic machinery of RNAPII ubiquitylation in response to transcriptional arrest.

Transcript elongation by RNAPII involves frequent pausing and stalling [65]. During transcriptional arrest, RNAPII backtracks and, through the help of the elongation factor TFIIS, is able to perform a hydrolytic cleavage of the RNA transcript in its active site [66]. This cleavage event allows RNAPII to re-establish its contact with the 3' end of the RNA. Although most arrested elongation complexes are likely to be rescued by the combined action of many elongation factors, a "last resort" mechanism, namely ubiquitylation and degradation, is used to ensure that transcription of genes is not blocked by an irreversibly-arrested polymerase. Poly-ubiquitylation and degradation of RNAPII was previously thought to occur specifically in response to DNA damage [67–69], but more recent experiments have shown that it is a general response to stalled or arrested elongation complexes [52, 64]. Interestingly in the absence of TFIIS, a functional RNAPII ubiquitylation system is required for yeast to survive. Conversely, in the absence of Def1 (RNAPII degradation factor 1), TFIIS becomes essential [70]. These observations suggest that the rescue of polymerase undergoing transcriptional arrest requires either the activity of TFIIS to re-activate backtracked RNAPII, or RNAPII ubiquitylation and degradation to completely remove the arrested RNAPII.

Using an *in vitro* system, Somesh *et al.* found that RNAPII in a purified ternary elongation complex (i.e., RNAPII, DNA and RNA) is a much better substrate for ubiquitylation than the same RNAPII either in a free form or when it is associated with DNA alone [71]. The specificity for the elongating polymerase is determined by its CTD phosphorylation state. In yeast, Ser2-phosphorylated CTD recruits the HECT E3 ligase Rsp5 which mono-ubiquitylates Rpb1; this is followed by extension with K48-linked polyUb catalyzed by a different E3 ligase, the Elc1/Cul3 complex [72, 73]. The requirement for two distinct E3 ligases implies that ubiquitylation of RNAPII must be tightly regulated to avoid unscheduled degradation. It is possible that mono-ubiquitylation of RNAPII might initially enable rescue mechanisms that do not involve proteolysis. In yeast, the Ubp3 deubiquitylating enzyme removes mono- or polyUb from ubiquitylated RNAPII [74]. How these enzymes coordinate to facilitate optimal transcription is an open question.

4. The UPS and mRNA export

Components of the UPS are involved in the nuclear export of mature transcripts. In particular, two ubiquitin ligases from the HECT family, Rsp5 and Tom1, control assembly and disassembly of mRNA export complexes (Fig. 3) [75–79].

The THO/TREX complex travels with RNAPII and couples transcription elongation to mRNA export [80]. Hpr1, a key component of this complex, is ubiquitylated by Rsp5 and targeted for degradation by the proteasome [79]. In yeast, either deletion of *HPR1* or stabilization of Hpr1 by inactivation of Rsp5 leads to defects in general mRNA export. This suggests that both Hpr1-dependent assembly and disassembly of the THO/TREX complex are important for efficient export. Ubiquitylation of Hpr1 depends on active transcription

[81]. PolyUb attached to Hpr1 enhances recruitment of the mRNA export receptor Mex67, which contains a UBA (Ub-associated) Ub-binding domain [82, 83]. This interaction shields Hpr1 from targeting to the proteasome. Interestingly, the same UBA domain also interacts with FG nucleoporins at the nuclear pore complex (NPC) [84]. Because polyUb and nucleoporins compete for binding to the UBA domain, once Mex67 delivers the transcripts to the pore, ubiquitylated Hpr1 will lose its protection and be rapidly degraded. Thus, the UPS not only links active transcription to mRNA export, but also controls the timing of dissolution of the two processes.

Recently, ubiquitylation of a mRNA-adaptor protein was found to participate in mRNP surveillance (Fig. 3) [83]. The mRNA export receptor Mex67 associates with nascent transcripts through adaptor proteins such as Nab2 and Yra1. Whereas Nab2 shuttles between nucleus and cytoplasm, Yra1 remains nuclear [85]. Iglesias *et al.* found that Yra1 is mono or oligo-ubiquitylated by the E3 Ub ligase Tom1, and that ubiquitylation of Yra1 promotes dissociation of Yra1 from the Nab2-mRNP complex to facilitate release of the mRNP into cytoplasm [83]. Ubiquitylation of Yra1 likely occurs at a late step of mRNA export. Genetic interactions between Yra1, Tom1, and components of the nuclear pore complex suggest that together they may serve as a checkpoint for properly processed mRNPs.

In addition, the SAGA co-activator is another important player in transcription-coupled mRNA export. SAGA interacts with the export machinery through the TREX2 complex, which is located at the inner face of the NPC (Fig. 3). Through this and other interactions, SAGA can mediate anchoring of actively-transcribed genes to the NPC [86], thus dramatically enhanced the efficiency of export. As mentioned in Section 2 of this review, the 19S RP contributes to SAGA recruitment both *in vitro* and *in vivo*, therefore enhancing transcription elongation and mRNA processing indirectly.

5. Conclusions

The UPS regulates gene expression through numerous proteolytic and non-proteolytic mechanisms. Due to space limitation, we did not discuss the important non-proteolytic functions of histone ubiquitylation in regulating chromatin dynamics and DNA damage repair. These topics are covered in several excellent reviews [10, 87–89].

Because ubiquitylation is a reversible post-translational modification, it is well suited to regulate dynamic processes like transcription. Among the many questions that remain in the field, it seems to us that two general issues are most critical: How is the balance of ubiquitylation and deubiquitylation achieved and coordinated with other events in gene expression? What are the receptor proteins that recognize the mono- or polyUb signals? Elucidation of the answers is a major and exciting challenge for researchers with interests in either (or both!) the ubiquitin-proteasome or gene expression fields.

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Abbreviations

UPS	ubiquitin-proteasome system
Ub	ubiquitin

CP	core proteasome
RP	regulatory particle
APIS	ATPase independent of 20S
CTD	c-terminal domain
RNAPII	RNA polymerase II
ChIP	chromatin immunoprecipitation
UBA	ubiquitin-associated
NPC	nuclear pore complex

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Fig. 1. (A) A schematic of different types of ubiquitin modifications and their functions. Ubiquitin has seven lysines and an N-terminal amine, each of which can engage in polyUb chain formation. These diverse modifications are associated with a large variety of biological functions, some of which are still poorly understood. (B) A schematic of the 26S proteasome and its subcomplexes.

Fig. 2. The UPS regulates transcription via both proteolytic and non-proteolytic approaches. (A) Ubiquitylation and degradation of the activator facilitates promoter escape of RNA polymerase II in each round of transcription. AD, activation domain; DBD, DNA binding domain; UAS, upstream activating sequences; GTF, general transcription factor. (B) Mono-ubiquitylation of the activator facilitates transcription by preventing APIS from stripping the activator off DNA. (C) Interactions between the activator, proteasome and SAGA stabilize the ternary complex to facilitate transcription.

Fig. 3.

The UPS regulates mRNA export in multiple ways. The SAGA complex, possibly recruited by the proteasome, can relocate the gene to the proximity of the NPC via the Sus1 subunit. Ubiquitylation of Hpr1 is coupled to transcription and facilitates recruitment of mRNA receptor Mex67. As part of the mRNA receptor complex, dissociation of Yra1 from mature transcript is dependent on mono-ubiquitylation.