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# Diverse Regulatory Mechanisms of Eukaryotic Transcriptional Activation by the Proteasome Complex

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**The life of any protein within a cell begins with transcriptional activation, and ends with proteolytic degradation. Intriguingly, the 26S proteasome complex, a non-lysosomal protein degradation machine comprising the 20S proteolytic core and 19S regulatory particles, has been implicated in intimate regulation of eukaryotic transcriptional activation through diverse mechanisms in a proteolysis-dependent as well as independent manner. Here, we discuss the intricate mechanisms of such proteasomal regulation of eukaryotic gene activation via multiple pathways.**

**Keywords** transcriptional activation, 26S proteasome, 19S regulatory particle

## INTRODUCTION

A large number of human diseases including various types of cancers are linked to abnormal gene regulation, and thus a detailed understanding of the proteins and regulatory mechanisms that dictate altered gene expression is a vital step towards developing drugs and therapies for maintaining normal cellular functions. In eukaryotes, gene regulation is largely controlled at the level of transcription, which can be divided mechanistically into different steps, such as preinitiation complex (PIC) formation, initiation, elongation, and termination. The recruitment of several general transcription factors (GTFs) such as TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, and RNA polymerase II (RNAPII) holoenzyme to the core promoter results in formation of the PIC assembly, and subsequently the initiation of transcription which is followed by elongation and finally termination (Roeder, 1996, 2005; Lee and Young, 2000).

Transcriptional initiation is an important regulatory step of gene expression. It is greatly stimulated by gene-specific activator proteins (activators) whose recognition sites are present within the promoter region. A typical activator contains a promoter-targeting region, which is often a sequence-specific DNA binding domain, and a distinct activation domain. A variety of studies (Ptashne, 1988; Ptashne and Gann, 1990, 1997; Roeder, 2005) indicates that activator interacts directly with one or more components of the transcription machinery to stimulate the assembly of GTFs for formation of the PIC at the core promoter. Thus, a large number of proteins must interact with the promoter DNA either directly or indirectly during transcriptional initiation or activation. How these interactions are precisely orchestrated in living cells to initiate transcription is not clearly known. Recently, a growing number of studies (Gonzalez *et al*., 2002; Métivier *et al*., 2003; Morris *et al*., 2003; Reid *et al*., 2003; Perissi *et al*., 2004; Zhu *et al*., 2004, 2007; Lee *et al*., 2005; Valley *et al*., 2005; Auld and Silver, 2006; Collins and Tansey, 2006; Kodadek *et al*., 2006; Pan *et al*., 2006; Rasti *et al*., 2006; Sulahian *et al*., 2006; Szutorisz *et al*., 2006; Zhang *et al*., 2006; Dentin *et al*., 2007; Ferdous *et al*., 2007; Laribee *et al*., 2007; Kinyamu and Archer, 2007; Lassot *et al*., 2007; Sikder and Kodadek, 2007; Archer *et al*., 2008; Bhat *et al*., 2008; Soond *et al*., 2008; Yi *et al*., 2008) have implicated the 26S proteasome, a highly versatile non-lysosomal protein degradation machine with a molecular chaperonin activity, in controlling and orchestrating the interactions of transcriptional initiation factors, and their localizations and abundances to regulate transcriptional initiation or activation through multiple pathways. Here, we review these diverse regulatory mechanisms of transcriptional activation by the proteasome.

## THE 26S PROTEASOME COMPLEX

The 26S proteasome is a non-lysosomal proteolytic machinery in eukaryotes (Glickman *et al*., 1998; Voges *et al*., 1999; Coux, 2002). It consists of a 20S proteolytic core particle (CP) and a 19S regulatory particle (RP). The 20S CP has a cylinder-like structure composed of a stack of two $\alpha$ and two $\beta$ rings

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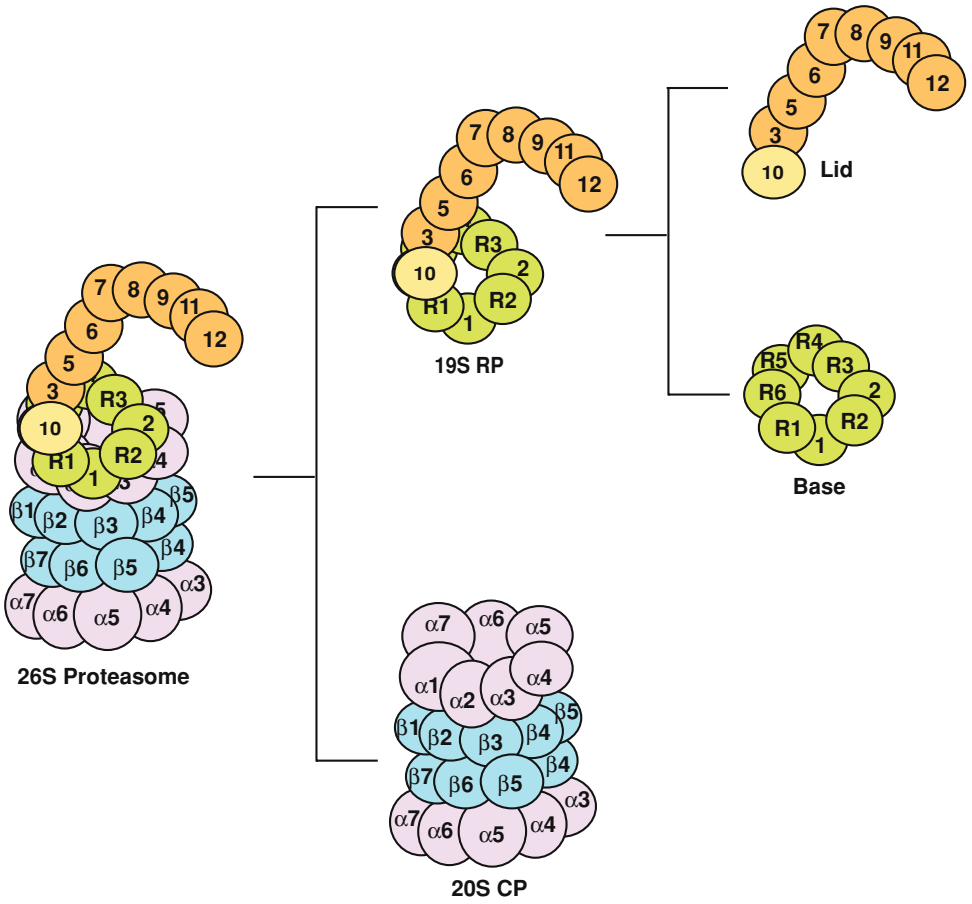


FIG. 1. Schematic representation of the 26S proteasome and its subunits. The proteasome is composed of two subunits, 19S RP (regulatory particle) and 20S CP (catalytic particle). The 19S RP bound to either or both ends of the 20S CP stimulates ATP-dependent degradation of K48-linked polyubiquitinated proteins. The 19S RP further consists of two subcomplexes, namely "base" and "lid". The Rpn proteins are denoted by the numbers, and the proteins Rpt1-Rpt6 are represented by R1-R6. Rpn10 appears to exist at the interface of the lid ans base subcomplexes (Glickman *et al*. 1998).

($\alpha$1–$\alpha$7 or $\beta$1–$\beta$7) in the order of $\alpha$ $\beta$ $\beta$ $\alpha$ (Figure 1). Three of the seven $\beta$-subunits are catalytically active, and are named by their substrate specificities as chymotrypsin-like ($\beta$5), trypsin-like ($\beta$2), and post-acidic or caspase-like ($\beta$1) (Dick *et al*., 1998; Groll and Clausen, 2003). The 19S RP comprises a "lid" of eight non-ATPases (Rpn3, Rpn5–Rpn9, Rpn11 and Rpn12) and a "base" of six ATPases (Rpt1–Rpt6) belonging to the AAA (ATPases associated witha variety of cellular activities) family, and two non-ATPase proteins (Rpn1 and Rpn2) Glickman *et al*., 1998. The base ATPases contain a C-terminal hydrophobic-tyrosine-X motif (HbYX) (Smith *et al*., 2007). These C-terminal residues of the base ATPases dock into the pockets of the 20S $\alpha$ rings upon ATP binding, and forms the 26S proteasome complex (Smith *et al*., 2007). Further, binding of ATP to the base ATPases serves as a "key" to open the gate of the 20S CP to allow the entry of protein substrates for degradation (Smith *et al*., 2007; Horwitz *et al*., 2007a).

The 26S proteasome degrades proteins marked by a chain of more than four ubiquitin (Ub) molecules. Ubiquitin is attached to cellular proteins through formation of an isopeptide bond between the C-terminus of ubiquitin and a lysine (K) side chain of the target protein by sequential actions of E1 activating, E2 conjugating, and E3 ligase enzymes (Pickart and Eddins, 2004; Pickart and Fushman, 2004; Wang *et al*., 2006). This ubiquitin molecule is further conjugated to other ubiquitin molecules through its K residues to generate structurally distinct polyubiquitin chains (Pickart and Eddins, 2004; Pickart and Fushman, 2004; Wang *et al*., 2006). The ubiquitin molecule has seven K residues (K-6, K-11, K-27, K-29, K-33, K-48, and K-63), and thus, seven isopeptide linkages can be formed with these K residues. It is the nature of ubiquitin linkage which determines the fate of a protein. K48-linked polyubiquitin chain constitutes the "kiss of death" for a protein (Chau *et al*., 1989; Finley *et al*., 1994; Wang *et al*., 2006). Although most of the proteins are

targeted for proteasomal degradation by K-48 linked polyubiquitin chain, it has been recently demonstrated that polyubiquitin chain linked through K-63 may also serve as a signal for proteasome-dependent degradation of some proteins (Kim *et al*., 2007). The existence of forked-ubiquitin chains containing all possible isopeptide linkages has also been demonstrated (Kim *et al*., 2007). These chains are resistant to proteolytic degradation by the proteasome, and play important roles in signaling and other regulatory pathways (Spence *et al*., 1995; Deng *et al*., 2000; Hicke and Dunn, 2003; Pickart and Cohen, 2004; Kim *et al*., 2007).

The lid of the 26S proteasome complex associates with polyubiquitin chain (Voges *et al*., 1999; Coux, 2002; Verma *et al*., 2004; Seong *et al*., 2007). Subsequently, the base unfolds the substrate protein in an ATP-dependent manner, and then translocates it into the central chamber of the 20S CP for proteolysis (Larsen and Finley, 1997; Voges *et al*., 1999; Navon and Goldberg, 2001; Coux, 2002; Smith *et al*., 2005). Through this mechanism, the 26S proteasome degrades a variety of oncoproteins, transcription factors, cell cycle specific cyclins, cyclin-dependent kinase inhibitors, ornithine decarboxylase, and other key regulatory cellular proteins (Hilt and Wolf, 1996; He *et al*., 1998), and, thus, regulates many important cellular processes directly or indirectly. However, the 26S proteasome complex has also been implicated *in vitro* to degrade many unfolded proteins without ubiquitin conjugation (Tarcsa *et al*., 2000). Furthermore, small peptides and certain denatured or oxidized proteins are degraded by the 20S proteasome in an ATP-independent manner *in vitro* (Li *et al*., 2007).

## THE 26S PROTEASOME COMPLEX IN TRANSCRIPTION

The 26S proteasome complex regulates the functions and fates of transcription factors in a highly responsive and coordinated manner. Among the key factors regulated by the proteasome or ubiquitin-proteasome system (UPS) are signal transducer (e.g. STAT and NF-kB), nuclear receptors (e.g. ER and GR), transcriptional activators (e.g. p53 and HIF-1$\alpha$), and a series of other transcription factors such as those involved in bone development (e.g. Runx2) and muscle regulation (e.g. MyoD). Thus, transcription and proteasomal degradation are linked together for regulation of gene expression.

To determine whether the proteasome is globally required for transcription, several research groups (Sikder *et al*., 2006; Dembla-Rajpal *et al*., 2004; Fleming *et al*., 2002) performed a genome-wide expression analysis in yeast. These studies have demonstrated that chemical or genetic impairment of the proteasome leads to a rapid transcriptional upregulation of genes involved in protein degradation, stress response, and mitochondrial function. On the other hand, genes for histone synthesis, mating, and amino acid metabolism or synthesis are downregulated. Thus, the 26S proteasome seems to regulate transcription of a large number of genes. Indeed, the temperature-sensitive inactivation of the proteasome alters the levels of $\sim$70% genomic transcripts at least by 2-fold (Sikder *et al*., 2006). It is thus expected that a large number of human genes, like in yeast, would also be regulated by the proteasome. Indeed, Poulaki *et al*. (2007) have recently demonstrated the impact of the proteasome inhibitor Bortezomib (also known as Velcade) on transcription profiling in retinoblastoma cell lines. They found that stress-response proteins, several proteasomal subunits, heat-shock proteins, pro-apoptotic proteins, cell-cycle regulators, cytokines, and a set of transcription factors are upregulated upon Bortezomib-treatment of retinoblastoma cell lines. On the other hand, Bortezomib also downregulates anti-apoptotic and adhesion molecules in retinoblastoma cell lines (Poulaki *et al*., 2007). Similarly, the gene-expression analysis in Bortezomib-treated pancreatic adenocarcinoma, classical Hodgkin lymphoma, multiple myeloma, and primary neuronal cells revealed differential expressions of genes involved in a variety of key cellular pathways including cell cycle and apoptosis (Yew *et al*., 2005, Mulligan *et al*., 2007, Zhao *et al*., 2008, Tang *et al*., 2008). Thus, the proteasome plays an important role in transcriptional regulation of a large number of human genes involved in important cellular processes, and has, therefore, become an attractive therapeutic target for treatment of a broad range of human diseases including cancer. Several proteasome inhibitors (e.g., Carfilzomib, Salinosporin A, PR-047, and PR-957) are now in clinical trials for treatment of various types of cancers (Montagut *et al*., 2006; Voorhees and Orlowski, 2006; Bennett and Kirk, 2008; Orlowski and Kuhn, 2008; Sterz *et al*., 2008). Bortezomib has already been approved in the USA for treating multiple myeloma and mantle cell lymphoma (a fast-growing cancer that begins in the cells of the immune system) (Orlowski and Kuhn, 2008).

The genome-wide expression analysis is not sufficient to differentiate direct transcriptional effect from the secondary effects of proteasome inactivation or inhibition, since the 26S proteasome is also involved in a variety of other functions within a cell (Tansey, 2004; Goldberg, 2007; Hanna and Finley, 2007). Thus, it is important to analyze the genome-wide location of the proteasome components in conjunction with transcription profiling. In view of this, two research groups (Auld *et al*., 2006; Sikder *et al*., 2006) performed a global genome-wide location analysis for the proteasome complex in yeast.These studies revealed that the 26S proteasome components are associated with the majority of genes, most of which are strongly correlated with expression levels and association of RNAPII. For example, highly transcribed genes including those involved in protein translation and glycolysis are associated with the proteasome (Auld *et al*., 2006; Sikder *et al*., 2006). Similarly, the 26S proteasome is also recruited to the lipid metabolism and mating type-specific genes (Auld *et al*., 2006; Sikder *et al*., 2006). The expression of similar sets of genes is altered in the proteasome mutants (Auld *et al*., 2006; Sikder *et al*., 2006). Thus, the global genome-wide location and expression analyses together revealed that the proteasome regulates transcription of a large number of genes within a cell.

Interestingly, the genome-wide location analysis in yeast demonstrated that several hundred genes are associated with either the 19S RP or 20S CP of the 26S proteasome (Auld *et al*., 2006; Sikder *et al*., 2006). Thus, the 19S RP and 20S CP seem to play independent functions at a certain set of genes. Consistent with this observation, Sulahian *et al*. (2006) have shown that transcription of several stress-responsive genes such as *HSP26*, *HSP104* and *GAD1* is regulated by the 19S RP independently of the proteolytic function of the 20S CP. On the other hand, the expression of the ribosomal protein genes is dependent on the proteolytic function of the 20S CP (Fleming *et al*., 2002; Dembla-Rajpal *et al*., 2004; Sikder *et al*., 2006). Consistent with these studies, Sikder *et al*. (2006) have further demonstrated that only ~900 genes are misregulated following treatment with 20S protease inhibitor MG132, and these genes account for ~23% of that (~70%) affected by inactivation of the proteasome. Thus, the 26S proteasome complex plays distinct functions in regulating transcription at different sets of genes in a proteolysis-dependent as well as independent manner. In the following sections, we discuss the proteolytic and non-proteolytic roles of the 26S proteasome in regulation of transcriptional activation or initiation, since this review is focused on the proteasomal regulation of transcriptional activation. However, the proteasome has also been implicated in regulation of transcriptional elongation (Ferdous *et al*., 2001, 2002; Ezhkova and Tansey, 2004; Collins and Tansey, 2006) and termination (Gillette *et al*., 2004; Collins and Tansey, 2006).

## THE PROTEOLYTIC ROLE OF THE 26S PROTEASOME IN REGULATION OF TRANSCRIPTIONAL ACTIVATION

The 26S proteasome complex regulates transcriptional activation in a proteolysis-dependent manner through multiple mechanisms including the localizations, abundances and activities of transcriptional activators and their interactions with promoters and coactivators as discussed below.

### Regulation of Activator Localization by the 26S Proteasome

The transcriptional activators need to be localized into the nucleus to stimulate transcription. Thus, activator localization regulates gene expression (Figure 2A). Such a simple regulatory mechanism is achieved by the UPS. For example, nuclear factor (NF)-kB, a transcriptional activator, is present in the cytoplasm where it interacts with the inhibitor protein, IkB. Following inflammation, IkB is phosphorylated, subsequently polyubiquitinated, and finally degraded by the 26S proteasome (Palombella *et al*., 1994; Tian and Matouschek, 2006). Destruction of IkB releases NF-kB to move into the nucleus for transcriptional activation of the target genes (Figure 2A). A similar mechanism is also used to regulate the localization of yeast transcriptional activator Spt23 that controls transcription of genes involved in fatty-acid metabolism (Hoppe *et al*., 2000; Tian and Matouschek, 2006). Following its synthesis, Spt23 is localized on the outer membrane of endoplasmic reticulum. When the levels of fatty acids in endoplasmic reticulum drop, Spt23 is polyubiquitinated, and detached from the membrane of endoplasmic reticulum by the 26S proteasome. Spt23 then enters into the nucleus to activate transcription of appropriate target genes (Figure 2A). Thus, the UPS is engaged in eliciting an efficient transcriptional activation in response to signals/stimuli. However, whether such a regulatory mechanism is restricted to a few or a large number of transcriptional activators remains to be elucidated.

### Regulation of Activator Abundance by the 26S Proteasome

In addition to maintaining the localization of activator, the UPS also keeps the levels of many transcriptional activators low via polyubiquitin-mediated proteasomal degradation. Such a low level of activator seems to maintain lower expression of target genes (Figure 2A). Thus, a reduction in polyubiquitination/proteolysis in response to some signaling pathways or changes in cellular conditions serves to regulate expression of a set of target genes. Such regulation is well-documented for p53 (Haupt *et al*., 1997; Alarcon-Vargas and Ronai, 2002; Lukashchuk and Vousden, 2007), an important transcriptional activator of genes involved in cell cycle arrest, stress response, DNA repair, apoptosis, etc. When cellular concentration of p53 is elevated, it is polyubiquitinated by Mdm2 (a RING finger E3 ligase), and then degraded by the 26S proteasome. Under stress conditions, p53 is phosphorylated by kinases such as ATM and ATR at the N-terminus. Such a phosphorylation prevents the interaction of p53 with Mdm2, thus leading to the stabilization and accumulation of p53 for transcriptional activation of its target genes (e.g. p21). Like p53, hypoxia-inducible factor-1$\alpha$ (HIF-1$\alpha$) is also regulated by the 26S proteasome (Maxwell *et al*., 1999; Zhu and Bunn, 2001; Baek *et al*., 2005). Under normoxic conditions, prolines 402 and 564 of HIF-1$\alpha$ are hydroxylated to allow its binding to von Hippel Lindau protein (VHL). Subsequently, VHL in conjunction with elongin B, elongin C and Cul2 functions as an E3 ubiquitin ligase, and polyubiquitinates HIF-1$\alpha$ for degradation by the 26S proteasome. HIF-1$\alpha$ is stabilized in the absence of its hydroxylation under hypoxic conditions, and binds to HIF-1$\beta$ to form an active complex that stimulates transcription by binding to the hypoxia response elements in the promoters of target genes. Like hypoxic condition, orexin (neuropeptide) has recently been shown to induce HIF-1$\alpha$ gene transcription under normoxic conditions, and consequently enhances HIF-1$\alpha$-mediated transcription of genes involved in glucose utilization, thus inducing food consumption and hunger (Sikder and Kodadek, 2007). Such an orexin-mediated stimulation of HIF-1 activity is caused by increased transcription of the HIF-1$\alpha$ gene and simultaneous decrease in the proteasomal degradation of HIF-1$\alpha$ via down-regulation of VHL (Sikder and Kodadek, 2007). Like p53 and HIF-1$\alpha$, the abundances of several other transcriptional activators are regulated by the 26S proteasome complex. Thus, the 26S proteasome complex controls transcriptional activation of a set of genes by regulating the abundances of transcriptional activators.

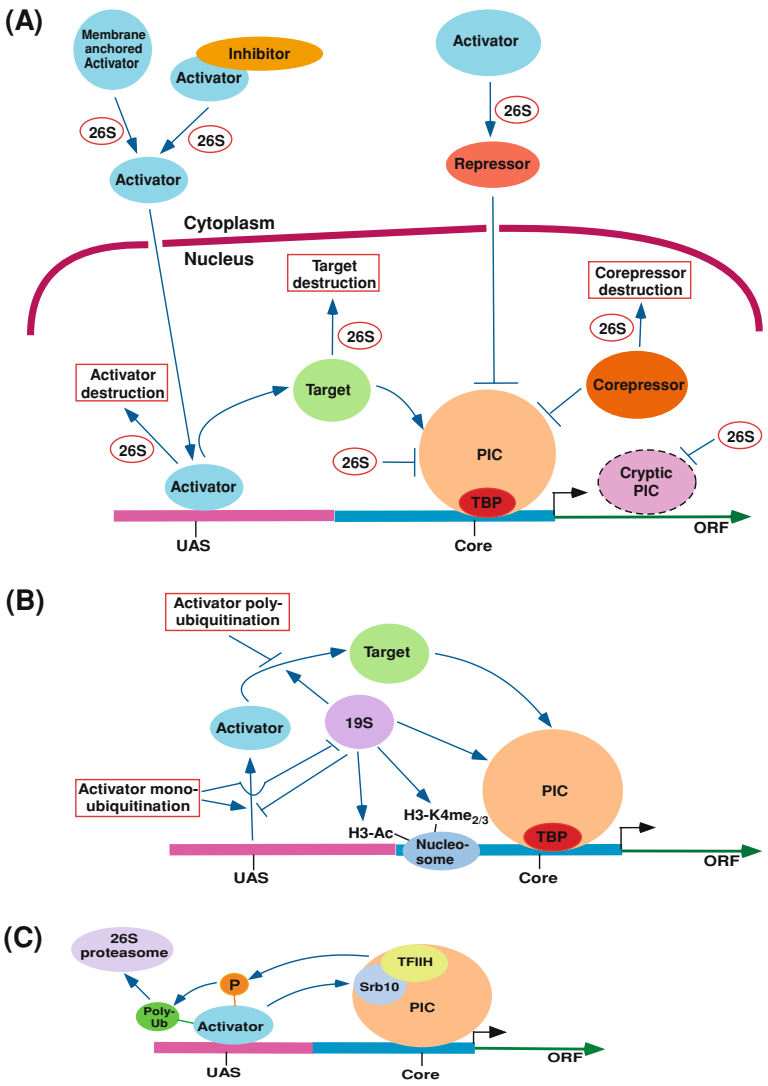


FIG. 2. Schematic diagrams representing multiple regulatory pathways of transcriptional activation by the proteasome in a proteolysis-dependent (A) as well as independent (B) manner. The proteasome regulates transcriptional activation by controlling the localizations, abundances and activities of transcriptional activators, and their interactions with coactivators (targets) and promoters. The proteasome also controls transcriptional activation by converting a transcriptional activator to a competitive repressor, destroying corepressor or cryptic PIC, and modulating histone covalent modifications. 26S, 26S proteasome; 19S, 19S RP; UAS, upstream activating sequence; core, core promoter element; ORF, open reading frame; PIC, pre-initiation complex; TBP, TATA-box-binding protein; H3-K4me$_{2/3}$, histone H3 lysine 4 di and trimethylation; and H3-Ac, histone H3 lysine acetylation. (C) A schematic diagram showing the destruction of an activator in a transcription-dependent manner. In the first round of transcriptional activation, activator stimulates the assembly of GTFs to form the PIC, and then the component(s) of the basal transcription machinery (e.g. Srb10 or TFIIH) facilitate phosphorylation of activator. Such a phosphorylation triggers K48-linked polyubiquitination of activator which is subsequently recognized and degraded by the 26S proteasome.

### Activator Destruction by the 26S Proteasome in a Transcription-Dependent Manner

As discussed above, the proteasomal degradation of transcriptional activators regulates transcription. Interestingly, several studies (Chi *et al*., 2001; Hirst *et al*., 1999; Muratani *et al*., 2005; Lipford *et al*., 2005) have demonstrated that the proteasomal degradation of transcriptional activator can also be triggered by transcription (Figure 2C). For example, Gcn4, a

yeast activator that is involved in amino-acid biosynthesis, is targeted for polyubiquitination in a phosphorylation-dependent manner. The phosphorylation of Gcn4 is mediated by Srb10, a component of RNAPII holoenzyme (Chi *et al.* 2001). Thus, both phosphorylation and polyubiquitination on Gcn4 occurs during the process of transcriptional activation. Polyubiquitinated-Gcn4 is then degraded by the 26S proteasome. Therefore, the basal transcription machinery marks an activator for its destruction. Such a transcription-dependent activator destruction seems to regulate gene activation. Indeed, transcription of the target genes of Gcn4 has been shown to be impaired when polyubiquitination of Gcn4 is prevented (Lipford *et al*., 2005). Like Gcn4, yeast activator Gal4 undergoes Srb10-dependent phosphorylation during active transcription (Hirst *et al*., 1999; Muratani *et al*., 2005), and the activated form of Gal4 has a short half-life (Muratani *et al*., 2005). Therefore, the function of Gal4 might be controlled by 26S proteasomal degradation. Indeed, Lipford *et al*. (2005) have demonstrated that the inhibition of the proteasome reduces transcription of the target genes of Gal4. Similarly, the proteasome-dependent proteolysis has been implicated in regulating the function of another inducible yeast activator system, Ino2/4 (Lipford *et al*., 2005). Together, these results lead to a model (Figure 2C) where proteasome-dependent proteolysis removes the "spent" activator, and resets the promoter. In this model, the first round of transcription does not require proteasome-dependent proteolysis of activator, but subsequent rounds of transcription are stimulated by polyubiquitination and the 26S proteasome-mediated degradation of the spent activator to allow recruitment of new activator. Thus, the activator's activity is required early in transcriptional activation. But, subsequent proteasomal degradation of activator promotes completion of transcription. However, such a transcription-dependent proteasomal degradation of activator is restricted to the transcriptional activators with acidic, but not proline or glutamine-rich, activation domain (Salghetti *et al*., 2000; Chi *et al*., 2001; Tansey, 2001). Further, Srb10-dependent phosphorylation does not always lead to polyubiquitination-dependent proteasomal degradation of transcriptional activators. For example, Srb10-dependent phosphorylation promotes rapid exclusion of multistress response activator Msn2 (Martinez-Pastor *et al*., 1996) from nucleus, but not its proteasomal degradation, and thus restricts Msn2 to regulate transcription (Chi *et al*. 2001; Tansey, 2001).

Like yeast activators, several nuclear receptors (also known as steroid receptors) in human are polyubiquitinated and subsequently degraded by the 26S proteasome in the course of their nuclear activities (Métivier *et al*., 2003; Reid *et al*., 2003; Nawaz and O'Malley, 2004; Valley *et al*., 2005; Alarid, 2006; Kinyamu and Archer, 2007; Kinyamu *et al*., 2008). Such a turnover of nuclear receptors is essential for tight regulation of target gene-activation (Métivier *et al*., 2003; Reid *et al*., 2003; Nawaz and O'Malley, 2004; Alarid, 2006). For example, estrogen receptor (ER$\alpha$) that performs crucial functions in normal reproduction is polyubiquitinated, and then degraded by the 26S proteasome during transcriptional activation in response to estradiol, leading to the cycling of ER$\alpha$ on promoter (Métivier *et al*., 2003; Reid *et al*., 2003; Nawaz and O'Malley, 2004; Alarid, 2006). Inhibition of the 26S proteasome lowers transcription of ER target genes (Métivier *et al*., 2003; Reid *et al*., 2003; Nawaz and O'Malley, 2004; Alarid, 2006). Degradation of ER$\alpha$ causes disassembly of the active transcription complex. Thus, the 26S proteasome-mediated cycling of ER$\alpha$ regulates disassembly and reassembly of the active transcription complex at the promoter. Similar to Gcn4, ER$\alpha$ undergoes proteasome-mediated turnover by basal transcription factors. The Cdk7 subunit of TFIIH phosphorylates S118 within AF-1 (activation function domain 1) transcriptional activation domain of ER$\alpha$ (Métivier *et al*., 2003; Reid *et al*., 2003; Nawaz and O'Malley, 2004; Alarid, 2006). This modification is required for recruitment of ubiquitin ligase and 19S RP for polyubiquitination and subsequent degradation of ER$\alpha$. Thus, basal transcription machinery plays an important role in activator turnover or disassembly of the transcription complex (Figure 2C). Similarly, the role of serine phosphorylation within AF-1 of PR (progesterone receptor) and RAR$\gamma$2 (retinoic acid receptor gamma 2) has been implicated in signaling the 26S proteasome-mediated degradation (Nawaz and O'Malley, 2004; Alarid, 2006).

Although several studies (Métivier *et al*., 2003; Reid *et al*., 2003; Nawaz and O'Malley, 2004; Alarid, 2006) have established a direct mechanistic connection between ER$\alpha$ degradation and transcriptional activity, Valley *et al*. (2005) have intriguingly demonstrated that proteolysis of ER$\alpha$ can be mechanistically separated from transcriptional activity. The proteolysis of ER$\alpha$ is regulated by the occupancy of ubiquitin ligase that is dependent on S118 phosphorylation of AF-1 transactivation domain. Further, S118 phosphorylation of AF-1 facilitates recruitment of coactivators and basal transcription machinery to form the active PIC by AF-2 (activation function domain 2) transactivation domain. The mutation (S118E-ER$\alpha$) that mimics the charge modification of phosphorylation enhances ligand-induced transcription along with recruitment of coactivator and basal transcription machinery. On the other hand, the S118A-ER$\alpha$ mutant that does not mimic charge modification significantly impairs recruitment of coactivator and basal transcription factors, hence greatly reducing transcription. Interestingly, both S118E- ER$\alpha$ and S118A-ER$\alpha$ mutants are unable to recruit ubiquitin ligase, and, hence, are protected from the 26S proteasomal degradation. Thus, S118 phosphorylation, but not charge modification, is specifically required for recruitment of ubiquitin ligase for polyubiquitination and subsequent degradation of ER$\alpha$. Therefore, S118 residue within the AF-1 region of ER$\alpha$ plays dual, but separate, functions in regulating the stability or proteolysis of ER$\alpha$ and its transcriptional activity. Thus, the destruction of ER$\alpha$ can be disengaged from its transcriptional activity.

Like ER$\alpha$, glucocorticoid receptors (GR) are also tightly regulated by the 26S proteasome (Deroo *et al*., 2002; Nawaz and O'Malley, 2004; Stavreva *et al*., 2004; Alarid, 2006). However, unlike ER-regulated genes, the expression of GR-target genes is

upregulated when the proteolytic activity of the 26S proteasome is impaired either genetically or chemically. Thus, cycling of GR by the 26S proteasome does not seem to be a key regulatory mechanism of GR-regulated gene activation, although the destruction or disassembly of receptor/transcription complex has been suggested as an important mechanism in controlling gene expression. Interestingly, a very recent study by Kinyamu and Archer (2007) has demonstrated that histone H3 K4 trimethylation, a covalent modification mark on histone for stimulated transcription, is elevated following inhibition of the proteasome at GR-regulated genes. Further, proteasomal inhibition increases the global level of phosphorylated-RNAPII as well as its association with target genes. These changes account for upregulation of GR-dependent gene expression following inhibition of the proteasome. Thus, the turnover of nuclear receptor by the 26S proteasome also regulates transcription by modulating histone covalent modification as well as recruitment of RNAPII.

Interestingly, recent works (Nalley *et al*., 2006, Yao *et al*., 2006) have shown that the proteasome-mediated degradation is not a general requirement for functions of all transcriptional activators. For example, Yao *et al*. (2006) have shown that a drosophila heat shock factor (HSF), the transcriptional activator of *HSP70*, is stably bound to the promoter following heat shock, and exchanges rapidly only under non-heat shock condition. Similarly, the activator Gal4 has been recently shown to be stably associated with promoter DNA under inducing condition, and is not regulated by the proteolytic function of the proteasome (Nalley *et al*., 2006), in contrast to the previous studies (Hirst *et al*., 1999; Lipford *et al*., 2005; Muratani *et al*., 2005). On the other hand, GR exchanges rapidly between nucleoplasm and a synthetic tandem array of its DNA response element in living cells, supporting active turnover mechanism to ensure the binding of fresh transcriptional activators to start new rounds of transcription. Thus, UPS-mediated turnover of activator can apply to some but not all transcriptional activators. However, why the 26S proteasome selectively regulates the functions of some transcriptional activators is not yet clearly understood.

## Targeted Proteolysis of Corepressor by the 26S Proteasome

Corepressor inhibits formation of the PIC assembly, and hence downregulates transcriptional activation. Thus, targeted degradation of corepressor by the 26S proteasome would stimulate transcription. Indeed, the 26S proteasome has been shown to be involved in targeted proteolysis of corepressor in cell-specific regulation of gene activation (Figure 2A) (Zhang *et al*., 1998). For example, cell-specific repression by nuclear receptor is correlated with the level of nuclear receptor corepressor (N-CoR) protein. The 26S proteasome regulates the level of N-CoR via mSiah2 that is expressed in a specific type of cell. mSiah2 interacts with N-CoR, and targets it to the 26S proteasome-mediated degradation, hence suppressing repression of the tissue-specific target genes (Zhang *et al*., 1998). Thus, targeted proteolysis of the corepressor complex by the 26S proteasome provides a mechanism for cell-specific regulation of gene activation. Importantly, multiple nuclear receptors function as repressors, and are required for brain function and development. Thus, 26S proteasome-mediated degradation of corepressor or N-CoR appears to play crucial roles in development. Similarly, the 26S proteasome is involved in degradation of several other transcriptional corepressors. For example, the corepressor SnoN in the TGF-$\beta$ (transforming growth factor-$\beta$) signaling pathway is degraded by the 26S proteasome complex (Bonni *et al*., 2001; Zhang *et al*., 2002). TGF-$\beta$ is an important superfamily of cytokines, and transduces signal through transmembrane Ser/Thr kinase receptors that regulate Smad proteins to mediate downstream signals. In the presence of TGF-$\beta$ signaling, Smurf2 (an E3 ubiquitin ligase) interacts with Smad2 as well as the transcriptional corepressor SnoN. Such an interaction network allows Smurf2 to target SnoN for ubiquitin-mediated proteasomal degradation, subsequently leading to the activation of target genes (Bonni *et al*., 2001; Stroschein *et al*., 2001; Zhang *et al*., 2002; Tan *et al*., 2008).

## Targeted Proteolysis of Coactivator by the 26S Proteasome

As mentioned above, activator stimulates PIC formation (and hence transcription) by interacting with one or more components of the transcription machinery, termed as target or coactivator. The 26S proteasome complex has been shown to influence the association of transcriptional activators with their targets or coactivators by degradation (Figure 2A). For example, the ubiquitin ligase, RLIM (RING finger LIM-interacting protein), interacts with a complex of transcriptional activator, LIM (which plays pivotal roles in neuronal development), and its coactivator CLIM (cofactor of LIM) on DNA (Ostendorff *et al*., 2002). RLIM then targets CLIM for polyubiquitin-mediated proteasomal degradation (Ostendorff *et al*., 2002). Such a destruction of coactivator facilitates the entry of a new coactivator to interact with promoter-bound activator for reprogramming transcriptional response. Similarly, the 26S proteasome degrades the coactivator, TORC2 (transducer of regulated CREB activity 2), and subsequently alters transcription of the target genes (Dentin *et al*., 2007, 2008). TORC2 participates in CREB (cAMP response element-binding protein)-dependent transcription of gluconeogenic genes. However, when the gluconeogenic pathway is switched off under low level of glucose or ATP, a salt-inducible kinase 2 (SIK2) phosphorylates TORC2 at Ser-171. This phosphorylation signals cytoplasmic export of TORC2 which is subsequently targeted for degradation by the 26S proteasome. Like CLIM and TORC2, the transcriptional coactivator p300 is also degraded by the 26S proteasome to regulate transcriptional activation (Poizat *et al*., 2000; Li *et al*., 2002; Brouillard and Cremisi, 2003). The p300 coactivator plays crucial roles in cell differentiation and signal transduction pathways. In mouse F9 cells, p300 plays an important transcriptional regulatory function in RA (retinoic acid)-induced differentiation (Brouillard and Cremisi, 2003). During RA-induced

F9 differentiation, the p300 coactivator is degraded by the 26S proteasome complex to regulate expression of genes involved in differentiation (Brouillard and Cremisi, 2003). Further, the proteasome-mediated degradation of p300 has been reported to occur in cardiac cells. Transcription of the target genes is impaired when cardiac cells are treated with the anticancer agent, Doxorubicin (Poizat *et al*., 2000). Similarly, sustained sodium butyrate treatment can induce the proteasome-mediated degradation of p300 to repress transcription of target genes (Li *et al*., 2002). Together, these studies have demonstrated that the 26S proteasome complex participates in degradation of transcriptional coactivators, hence regulating transcription of their target genes.

### Disassembly of the PIC by the 26S Proteasome

Apart from degrading the transcriptional activator, coactivator or corepressor, the 26S proteasome is also involved in turnover of the PIC assembly to regulate gene activation. For example, a recent study (Szutorisz *et al*., 2006) has elegantly demonstrated that the 26S proteasome destabilizes the PIC formed at the cryptic promoter in the ES (embryonic stem) cells to prevent incorrect transcriptional initiation, hence minimizing transcriptional noise at genes that promote differentiation (Figure 2A). Further, the 26S proteasome has been implicated in maintaining the pluripotency (where tissue-specific genes are in a potentiated state, but ready for activation at later stages) by suppressing the stable binding of transcription factors and RNAPII to the intergenic sequences to restrict permissive transcription in ES cells (Szutorisz *et al*., 2006). Thus, the 26S proteasome disassembles transcriptionally active and/or cryptic PIC to regulate transcriptional activation or initiation (Figure 2A). Further, Horwitz *et al*. (2007b) have recently demonstrated the role of UPS in regulation of BRCA1-mediated transcription at the level of PIC formation. BRCA1, the breast and ovarian cancer-specific tumor suppressor, constitutes an active E3 ubiquitin ligase activity when it forms a heterodimer with BARD1 (Hashizume *et al*., 2001). In the absence of ubiquitin or BARD1, BRCA1 strongly activates transcription by enhancing the formation of the PIC assembly at the core promoter (Horwitz *et al*., 2006). However, BRCA1, RNAPII and TFIIE are ubiquitinated in the presence of BARD1, ubiquitin and charged E2, resulting in the destabilization of the PIC (Starita *et al*., 2005; Horwitz *et al*., 2007b). Such destabilization leads to the repression of transcriptional initiation of target genes (Starita *et al*., 2005; Horwitz *et al*., 2007b). Thus, UPS regulates transcriptional activation at the level of PIC formation (Figure 2A).

### Conversion of a Transcriptional Activator to a Competitive Repressor by the 26S Proteasome

The 26S proteasome has been implicated in repressing transcription by converting transcriptional activator to a competitive repressor through proteolysis (Figure 2A). Such a regulation of transcriptional activation has been well-documented for *Drosophila* Cubitus interruptus (Ci) and its vertebrate homologs Gli2 and Gli3 (Aza-Blanc *et al*., 1997; Sasaki *et al*., 1999; Tian *et al*., 2005; Pan *et al*., 2006; Zhang *et al*., 2006; Varjosalo *et al*., 2008). Ci activates transcription of wingless, and represses hedgehog (Aza-Blanc *et al*., 1997). The proteasome transforms a full-length Ci (Ci155) in cytoplasm into a fragment that is translocated into nucleus to act as a competitive repressor (Ci75) of hedgehog and other target genes (Aza-Blanc *et al*., 1997). Such a partial degradation by the proteasome is regulated by zinc finger region and K750 of Ci155 (Wang and Price, 2008). In the presence of hedgehog (a secreted protein) signaling, the proteolysis of Ci is inhibited, hence leading to the expression of target genes (Aza-Blanc *et al*., 1997). Thus, the proteasome plays a pivotal role in converting a transcriptional activator to a competitive repressor to regulate transcriptional activation of target genes in response to the changes in hedgehog signaling during development. Similarly, the vertebrate homologue Gli2 is also regulated by UPS (Varjosalo *et al*., 2008). The kinase, DYRK2 (dual-specificity tyrosine phosphorylation regulated kinase 2), phosphorylates Gli2 at Ser-385 and 1011, and induces its degradation by UPS (Varjosalo *et al*., 2008). Such degradation of Gli2 leads to the repression of target genes involved in sonic hedgehog signaling (Varjosalo *et al*., 2008). Thus, UPS plays a crucial role in converting a transcriptional activator to a competitive repressor or small peptide fragments to control transcriptional activation (Figure 2A).

## THE NON-PROTEOLYTIC ROLE OF THE 26S PROTEASOME IN REGULATION OF TRANSCRIPTIONAL ACTIVATION

In addition to the global genome-wide location and expression analyses (as mentioned above), several biochemical studies (Xu *et al*., 1995; Weeda *et al*., 1997; Makino *et al*., 1999; Yanagi *et al*., 2000; Chang *et al*., 2001; Sun *et al*., 2002) have also implicated the non-proteolytic function of the proteasome in transcriptional activation on the basis of interaction of the 19S RP with activator and components of the transcriptional activation machinery such as RNAPII (Rpb1), TFIID (TBP, TAF5), TFIIH (Tfb2), and Mediator (Med8). However, how the proteasome impacts transcriptional activation in a proteolysis-independent manner is not clearly understood. Several recent studies (Lee *et al*., 2005; Collins and Tansey, 2006; Flick *et al*., 2006; Nalley *et al*., 2006; Szutorisz *et al*., 2006; Baker and Grant, 2007; Ferdous *et al*., 2007; Laribee *et al*., 2007; Archer *et al*., 2008; Bhat *et al*., 2008) have shed light on the 19S RP regulation of transcriptional activation at the levels of activator-promoter/coactivator interaction, PIC formation, and histone covalent modifications as discussed below.

### Regulation of Activator–Promoter Interaction by the 19S RP

In the process of transcriptional activation, activator first binds to promoter, and then enhances formation of the PIC assembly by interacting with one or more components of the transcriptional initiation machinery to stimulate transcription.

Thus, the activator–promoter interaction plays a pivotal role in regulation of transcriptional activation. Recently, Ferdous *et al*. (2007) have demonstrated that the 19S RP controls transcription by destabilizing the activator–promoter interaction (Figure 2B). Such a destabilization of activator–promoter interaction is dependent on activation domain and ATP, but does not involve degradation or irreversible denaturation of protein. Intriguingly, when activator is monoubiquitinated, the destabilization of the activator–promoter interaction by the 19S RP does not occur. For example, Gap71, a Gal4 derivative with mutations in the DNA binding domain, is not monoubiquitinated, and is quickly dissociated from DNA in the presence of the 19S RP. On the other hand, Nalley *et al*. (2006) have recently demonstrated that Gal4 forms a highly stable and long-lived complex with promoter DNA under inducing conditions. Thus, Gal4 seems to be monoubiquitinated, and hence appears to be protected from the destabilization effect of the 19S RP under inducing conditions. However, further investigations are required to analyze monoubiquitination of Gal4 and how it stabilizes interaction of activator with promoter.

How does the 19S RP destabilize the activator–promoter interaction in the absence of monoubiquitination? In bacteria, ClpX, the homologue of Rpt ATPase, destabilizes the Mu protein–DNA complex by unfolding Mu (Levchenko *et al*., 1995; Kim *et al*., 2000). Such a denaturation of Mu occurs by its interaction through a specific sequence with ClpX (Levchenko *et al*., 1995; Kim *et al*., 2000). However, the unfolded-Mu protein rapidly folds back to the functionally active form to occupy its binding site on DNA again (Levchenko *et al*., 1995; Kim *et al*., 2000). Similar to the function of ClpX on the stability of Mu–DNA complex, the 19S RP might be unfolding activator in an ATP-dependent manner. Subsequently, the unfolded activator is stripped off from DNA, which may fold back to associate with DNA again like bacterial Mu protein. However, such a model remains to be elucidated.

## Stimulation of the Activator–Target/Coactivator Interactions by the 19S RP

As mentioned above, the activator–target/coactivator interaction stimulates formation of the PIC assembly, and hence transcription. Such a regulation of transcriptional activation has recently been shown to be further controlled by the 19S RP in a proteolysis-independent manner for transcriptional activation of yeast *GAL* genes. At the promoters of *GAL* genes, the activator Gal4 recruits coactivator SAGA (Spt-Ada-Gcn5-acetyltransferase), a multi-protein complex with histone acetyltransferase (HAT) activity, which subsequently stimulates formation of the PIC assembly to initiate transcription (Bhaumik and Green 2001; Bhaumik *et al*., 2004). Thus, Gal4–SAGA interaction is an important step in regulation of *GAL* gene expression. Recently, Lee *et al*. (2005) have demonstrated that the 19S RP enhances the targeting of SAGA to the Gal4-driven promoter in an ATP-dependent manner, but independently of the 20S CP (Figure 2B). Further, their studies revealed that the base alone, but not lid, stimulates interaction of Gal4 with SAGA. Taken together, these data provide an elegant biochemical model for why the 19S RP is required for transcriptional activation of the *GAL* genes.

How does the 19S RP function in targeting SAGA to the gene promoter? It might be involved in facilitating the DNA-binding activity or HAT activity of SAGA. Indeed, Lee *et al*. (2005) have demonstrated that the 19S RP increases HAT as well as DNA-binding activities of SAGA. To regulate these activities of SAGA, the 19S RP might be physically interacting with it. To test this possibility, Lee *et al*. (2005) performed a co-immunoprecipitation assay which revealed that the 19S RP, indeed, physically interacts specifically with SAGA but not NuA4 (nucleosome acetyltransferase of H4) HAT complex. Such interaction has been further substantiated by genetic studies (Lee *et al*., 2005).

Although previous biochemical studies (Lee *et al*., 2005) have demonstrated the general function of ATP hydrolysis by the 19S RP to enhance physical interaction between Gal4 and SAGA, the role of each of the six 19S ATPase components in stabilization of Gal4–SAGA interaction is not yet known. Determination of the functions of these six 19S ATPases in enhancing Gal4-SAGA interaction will provide important biochemical insights on the 19S RP function in transcriptional activation. Further, it remained unknown until recently whether the 19S RP acts in a similar manner in regulation of mammalian transcriptional activation. However, a very recent study (Bhat *et al*., 2008) has indicated the role of the 19S ATPase in regulation of mammalian gene expression at the level of activator–coactivator interaction. This study shows recruitment of the 19S ATPase to promoter of the MHC class II gene, HLA-DR for stabilization of the interaction of class II transactivator (CIITA) with promoter to activate transcription. Intriguingly, after prolonged cytokine stimulation, there is a slow association of the 20S CP with the MHC Class II promoter. Recruitment of the 20S CP parallels the loss of CIITA from promoter. The binding of the 20S CP to promoter (where 19S RP is already present) leads to the reconstitution of the 26S proteasome. The 26S complex, thus formed, might be degrading CIITA, leading to the disassembly of transcription proteasome complex at the promoter (and hence leading to transcriptional termination). This model demonstrates the temporal regulation of transcription by the proteasome—where transcriptional initiation is aided in a non-proteolytic manner by the 19S RP alone, and transcriptional termination is brought about by the 26S proteasome complex.

## Inhibition of Activator–Target/Coactivator Interaction by Polyubiquitination in a Proteolysis-Independent Manner

As mentioned above, the activator–target interactions stimulate PIC formation, and hence lead to transcriptional activation. Thus, inhibition of activator-target/coactivator interaction would downregulate transcriptional activation. Such a downregulation of transcriptional activation has been shown to be caused by polyubiquitination in a proteolysis-independent

manner (Figure 2B). For example, ubiquitin ligase, SCFMet30, catalyzes polyubiquitination of yeast transcriptional activator Met4 that is required for expression of many genes involved in metabolism of sulfur amino acids. The polyubiquitinated-Met4 cannot recruit the partner protein (e.g., Cbf1) for transcription activation (Kaiser *et al*., 2000). Thus, polyubiquitination of transcriptional activators can directly regulate the interactions of activators with their coactivators, and hence transcriptional activation. Surprisingly, the ubiquitin chain that is attached to Met4 is polymerized through K48 of ubiquitin molecule, but is not targeted for degradation by the 26S proteasome (Flick *et al*., 2004). Thus, K48-linked polyubiquitin chain can have a regulatory role independently of proteolysis. Interestingly, a recent study (Flick *et al*., 2006) has demonstrated that ubiquitin-interacting motif protects polyubiquitinated-Met4 from the 26S proteasome-mediated degradation.

In contrast to polyubiquitination, monoubiquitination has been implicated in enhancing transcription. For example, Salghetti et al (2001) have demonstrated that the fusion of a ubiquitin molecule to the N-terminal tail of LexA-VP16 stimulates transcription. Thus, monoubiquitination, in contrast to polyubiquitination, seems to enhance activator–target/coactivator interaction to stimulate transcription in an unknown mechanism. However, a very recent study (Archer *et al*., 2008) has elucidated the mechanism for such monoubiquitination-mediated transcriptional stimulation using Gal4 activator. According to this model, the E3 ubiquitin ligase binds to the C-terminal region of the Gal4 activation domain, and subsequently ubiquitinates the DNA binding domain. Such an ubiquitination stabilizes Gal4–promoter interaction, resisting it from the proteasome-mediated destabilization.

### Stimulation of the PIC Formation by the 19S RP

Transcription is initiated by the assembly of GTFs at the promoter to form the PIC. However, if the GTFs are not properly assembled, it would lead to an abortive or inactive PIC which would not initiate transcription. Thus, a molecular chaperonin activity of the 19S RP is expected to play a pivotal role in forming the transcriptionally active PIC for efficient transcriptional initiation (Figure 2B). Indeed, a recent study (Szutorisz *et al*., 2006) has demonstrated recruitment of only 19S RP with molecular chaperonin activity but not 20S CP at the core promoter of active mammalian genes. Such recruitment of 19S RP possibly facilitates formation of the active PIC at the core promoter to initiate efficient transcription. However, such a model needs to be elucidated further. In this direction, we have performed a series of experiments to analyze the role of the 19S RP in formation of the transcription complex assembly at yeast promoter, using a ChIP assay. Our data (Malik and Bhaumik; unpublished) revealed that the 19S RP is essential for formation of the transcription complex assembly at the promoter for productive transcriptional initiation, consistent with previous biochemical data (Xu *et al*., 1995; Weeda *et al*., 1997; Makino *et al*., 1999; Yanagi *et al*., 2000; Chang *et al*., 2001; Sun *et al*., 2002) that demonstrated interaction of the 19S RP with components of the PIC assembly.

### Regulation of Histone Covalent Modifications by the 19S RP

The non-proteolytic function of the 19S RP has been implicated in regulation of histone H3 K4 and K79 methylation. For example, Ezhkova and Tansey (2004) have demonstrated that the 19S RP is essential for upregulation of histone H3 K4/79 methylation in a histone H2B ubiquitination-dependent manner. Further, the 19S RP has recently been shown to regulate histone H3 K4 methylation through its interaction with COMPASS (a histone methyltransferase in yeast) and Not4 (E3 ubiquitin ligase) of the CCR4/Not complex (Laribee *et al*., 2007). In addition to its role in regulation of histone H3 methylation, the 19S RP has also been implicated to regulate histone H3 acetylation. For example, Koues *et al*. (2008) have recently shown that 19S ATPase, Rpt6, interacts with CBP (CREB-binding protein)-HAT, and recruits it to the MHC class II promoter for histone H3 acetylation. Similarly, the 19S RP targets SAGA-HAT to the active gene, and acetylates histone H3 in yeast (Lee *et al*., 2005). These covalent modifications (i.e. histone H3 methylation and acetylation) stimulate transcription. Thus, the 19S RP regulates transcription through modulation of histone covalent modifications (Figure 2B).

## CONCLUDING REMARKS

In this review, we have discussed various regulatory pathways of transcriptional activation by the proteasome in a proteolysis-dependent and independent manner (Figure 2). Briefly, the proteasome regulates transcriptional activation by controlling the localizations, abundances and activities of transcriptional activators through proteolytic degradation. The proteasome also controls transcriptional activation by converting a transcriptional activator to a competitive repressor, destroying corepressor or cryptic PIC in a proteolysis-dependent manner. Furthermore, the proteasome has been implicated in degradation of some coactivators to facilitate the entry of new coactivators for reprogramming transcriptional response. On the other hand, the non-proteolytic function of the proteasome controls transcriptional activation by modulating histone covalent modifications and/or regulating the interactions of activators with coactivators and promoters. In addition, the proteasome stimulates formation of the PIC assembly for productive transcriptional activation in a proteolysis-independent manner. We have presented a handful of examples in support of these regulatory pathways. However, it is not yet known how general is each pathway of the proteasomal regulation of transcriptional activation, and what provides the signal or specificity for each mechanism. Moreover, why some but not all transcriptional activators/coactivators are destroyed by the proteasome to regulate transcriptional activation is not clearly understood. Further, there are many unanswered questions regarding the mechanistic details of the regulatory roles of

the proteasome in gene activation. For example, how does the proteasome interact with transcriptional activation machinery in living cells? What is the role of the proteasome in establishing the specific protein interaction network at the promoter during transcriptional activation *in vivo*? Do the six ATPase components of the proteasome play redundant or distinct functions in transcriptional activation? Thus, further investigations in this field, using biochemical, biophysical, genetic, and cell and molecular biological methodologies, will significantly advance our understanding of the regulatory mechanisms of gene activation by the proteasome. Such knowledge will definitely be very useful for designing as well as developing therapeutic agents to maintain normal cellular functions, since a growing number of human diseases are correlated with the proteasome at the level of transcription.



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