REVIEW: GENE EXPRESSION

Emerging Roles of Ubiquitin in Transcription Regulation

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Ubiquitin is a small protein that was initially found to function as a tag that can be covalently attached to proteins to mark them for destruction by a multisubunit, adenosine 5′-triphosphate–dependent protease called the proteasome. Ubiquitin is now emerging as a key regulator of eukaryotic messenger RNA synthesis, a process that depends on the RNA synthetic enzyme RNA polymerase II and the transcription factors that control its activity. Ubiquitin controls messenger RNA synthesis not only by mechanisms involving ubiquitin-dependent destruction of transcription factors by the proteasome, but also by an intriguing collection of previously unknown and unanticipated mechanisms that appear to be independent of the proteasome.

Transcription by the multisubunit enzyme RNA polymerase II (Pol II) of even the simplest eukaryotic protein-coding genes requires the participation of a large and diverse collection of proteins, including one or more DNA binding transcription activators or repressors, multiprotein Mediator-coactivator complexes, chromatin-remodeling and -modifying enzymes, and a cadre of general initiation and elongation factors (*1*, *2*). In addition, Pol II transcription is subject to multiple layers of regulation exerted both by recruitment to genes of Pol II, Mediator, and the general transcription factors and by reorganization of a gene's chromatin by enzymes such as histone acetyltransferases (HATs), histone deacetylases (HDACs), and adenosine 5′-triphosphate (ATP)–dependent SWI/SNF-like complexes. Now, in a series of recent studies, the protein-modifying peptide ubiquitin has been found to mediate an additional layer of Pol II transcription regulation and to do so by a variety of previously unknown and unanticipated mechanisms.

The Classic Ubiquitin-Dependent Proteolysis Pathway

Ubiquitin is a highly conserved, ~76–amino acid protein that was initially discovered as a macromolecular tag that could be covalently attached to certain target proteins to mark them for degradation by the proteasome, in what is now called the classic ubiquitin-dependent proteolysis pathway (*3*). A repetitive chain of ubiquitin molecules is conjugated to lysines in target proteins through a multienzyme cascade. In the first step, which is catalyzed by an E1 ubiquitin-activating enzyme, the COOH-terminus of ubiquitin is activated in an ATP-dependent step and covalently linked through a thioester bond to an active-site cysteine in the E1. In the second step, ubiquitin is transferred from the E1 to an active-site cysteine in 1 of more than 20 known E2 ubiquitin-conjugating enzymes. In the final step, which is accomplished by the action of an E3 ubiquitin ligase (*3*), ubiquitin is covalently linked by way of an isopeptide bond to a lysine in the target protein or to another ubiquitin molecule that has already been linked to the target protein. A target protein must be tagged with a multiubiquitin chain composed of at least four ubiquitins before it can be recognized and degraded by the proteasome. The E3 ubiquitin ligase appears to perform the singular task of recognizing, binding specifically to, and recruiting target proteins for ubiquitylation.

Cross talk among E3s. Although it has been known for some time that a number of Pol II transcription factors are regulated by ubiquitin-dependent proteolysis, an appreciation for the pervasive role of the classic ubiquitin-dependent proteolysis pathway in transcription regulation has come only within the last several years with the revelation that cells contain an unusually large collection of E3s with a growing list of Pol II transcription factors as their targets (Fig. 1 and Table 1). In addition, individual Pol II transcription factors can be regulated by multiple E3s, allowing integration of multiple signaling pathways at a common end point—ubiquitylation and/or destruction of a single transcription factor.

A prominent example of this type of regulation comes from evidence that defects in the cross talk between the E3s that govern the p53 tumor suppressor and hypoxia-inducible transcription factor HIF1 contribute to the vascularization and growth of of p53(−/−) tumors. Regulation of p53 levels in cells is accomplished largely through the activity of the p53 inhibitor Mdm2, a RING finger E3 that binds p53, inhibits its transcriptional activity, and promotes its ubiquitylation and degradation. Like p53, HIF1 is tightly regulated by ubiquitin-dependent proteolysis. When cells have a plentiful supply of oxygen, the α subunit of HIF1 is rapidly ubiquitylated and turned over in a reaction dependent on the multsubunit, Elongin BC-based VHL ubiquitin ligase complex (*4*–*7*). The interaction of HIF1α with the VHL complex requires oxygen-dependent hydroxylation of a proline residue in the HIFα oxygen-dependent degradation domain (ODD) by an Fe^{2+}-dependent prolyl hydroxylase (*8*, *9*) of the Egl-9 family (*10*, *11*). When cells are deprived of oxygen, HIF1α is no longer hydroxylated and targeted for ubiquitylation and destruction by the VHL E3. Under these conditions, cellular levels of HIFα rise, resulting in activation of transcription of hypoxically regulated genes, including vascular endothelial growth factor (VEGF). Although p53 does not appear to bind the VEGF promoter and directly control its transcription, p53 inhibits HIF-dependent VEGF transcription (*12*), and VEGF expression is enhanced in p53(−/−) cells (*13*).

A plausible mechanism for p53-dependent regulation of VEGF and other hypoxically regulated genes is suggested by results of recent studies indicating that HIF1α can interact with p53 (*12*, *13*) and, through p53, with the E3 ubiquitin ligase Mdm2 (*13*). As a consequence of that interaction, HIF1α could be be targeted for ubiquitylation and degradation not only by the VHL ubiquitin ligase, but also by p53-associated Mdm2 (*13*). Although confirmation of this model awaits biochemical reconstitution of p53- and Mdm2-dependent ubiquitylation of HIF1α, these findings, together with the observation that p53 itself can be activated by hypoxia, raise the possibilities that (i) p53 and Mdm2-dependent degradation of HIFα is part of a feedback mechanism that dampens the hypoxic response in normal cells, and (ii) en-

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hancement of the normal hypoxic response due to loss of p53-dependent HIFα degradation contributes to the increased vascularization and sustained growth of p53(−/−) tumors.

In a second example of E3 cross talk, the oncogenic transcription factor β-catenin is regulated by two E3s, one responsive to Wnt signaling and the other to activation of p53 by genotoxic stress and other signals. Previous studies had shown that, in the absence of a Wnt signal, β-catenin is rapidly phosphorylated in the cytoplasm of cells by glycogen synthase kinase 3β (GSK3β) (*14*) and ubiquitylated by an Skp1/cullin-1/F-box protein (SCF) E3 containing the F-box protein β-TRCP, which binds selectively to and recruits phosphorylated β-catenin for ubiquitylation (*15–18*). Upon Wnt signaling, phosphorylation and consequent ubiquitylation of β-catenin are blocked, and β-catenin is free to enter the nucleus, where it dimerizes with members of the TCF/LEF (T cell factor/lymphoid enhancer factor) family of transcription factors and activates its transcriptional program. Recently, β-catenin has also been shown to be regulated by ubiquitin-dependent proteolysis through a GSK3β-independent pathway that uses the RING finger E3 Siah-1 (*19*, *20*). This Siah-1 E3 is a previously unknown, Skp1-based E3 that represents an intriguing variation on the SCF E3 theme. In this multiprotein E3, Siah-1 replaces the Rbx1 subunit of classical SCF E3s, and Siah-1 interacting protein (SIP) links Siah-1 to Skp1 and the F-box protein Ebi (*19*). Interestingly, Siah-1 also functions as a single-subunit RING finger E3, which targets the oncogenic transcription factor c-myb (*21*). Expression of the multifunctional Siah-1 protein is induced by activated p53 (*22*, *23*), thus allowing integration of Wnt and p53-dependent signals in regulation of β-catenin.

Ubiquitin Expands Its Modus Operandi

Activation of transcription factors by ubiquitin-dependent processing. In a departure from the classic paradigm of ubiquitin-dependent destruction of target proteins, several Pol II transcription factors have now been shown to be proteolytically processed from inactive precursors to their active forms by ubiquitin-dependent action of the proteasome. The nuclear factor kappa B1 (NF-κB1) p105 and p100 precursors are processed into the active NF-κB p52 and p50 subunits by ubiquitylation and cleavage by the proteasome (*24*). Although the mechanism of this process remains controversial (*25–28*), evidence suggests that the $SCF^{\beta\text{-}TRCP}$ E3 can target the p105 and p100 COOH-terminal domains for ubiquitylation and degradation (*25*), leaving their transcriptionally active NH_2-terminal p52 and p50 domains to unite with their Rel partners and reconstitute the intact, active NF-κB heterodimer. Other results suggest, however, that ubiquitylation directed by $SCF^{\beta\text{-}TRCP}$ may target p105 for complete degradation (*29*), whereas p105 processing is accomplished cotranslationally (*27*), through another, as yet unknown, proteasome-dependent pathway (*29*).

In addition to NF-κB, two *Saccharomyces cerevisiae* transcription factors, SPT23 and MGA2, which play key roles in activation of expression of genes required for synthesis of polyunsaturated fatty acids, have recently been shown to be regulated by ubiquitin-dependent processing by the proteasome (*30*). When yeast are grown in media rich in polyunsaturated fatty acids, SPT23 and MGA2 are synthesized as inactive precursors that remain anchored by their COOH-terminal tails to the membrane of the endoplasmic reticulum. When yeast are deprived of polyunsaturated fatty acids, the inactive SPT23 and MGA2 precursors are ubiquitylated by the HECT domain E3 Rsp5 and processed by the proteasome, releasing them from the endoplasmic reticulum and allowing their translocation to the nucleus, where they activate expression of genes essential for growth of yeast deprived of polyunsaturated fatty acids. Although these two examples are presently the only reported cases of ubiquitin-dependent processing of transcription factors from inactive to active forms, it is likely that additional examples of this ubiquitin-dependent transcriptional regulatory mechanism will surface in the future.

Ubiquitin-dependent transcription regulation without proteolysis. In addition to its roles in directing degradation or processing of target proteins by the proteasome, ubiquitin has been found to function by an apparently proteasome-independent mechanism in a small but growing number of cases (*31*) in processes including endocytosis, histone

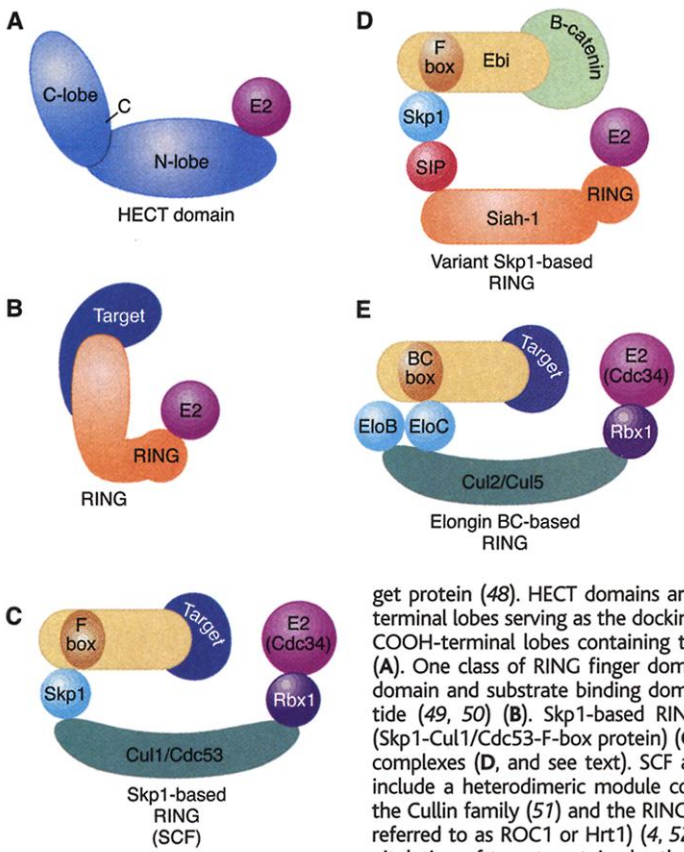

Fig. 1. A growing cast of E3 ubiquitin ligases function in the regulation of Pol II transcription. E3s with known roles in Pol II transcription include members of the families of HECT domain E3s, RING finger domain E3s, and the structurally related multiprotein Skp1- and Elongin BC-based E3s. HECT and RING finger domains serve as docking sites for E2 ubiquitin-conjugating enzymes (*46*, *47*). Unlike RING finger domains, HECT domains contain a catalytic cysteine residue that accepts ubiquitin from the E2 and transfers it to the target protein (*48*). HECT domains are bilobal, with their NH_2-terminal lobes serving as the docking site for the E2 and their COOH-terminal lobes containing the catalytic cysteine (*46*) (**A**). One class of RING finger domain E3s include the RING domain and substrate binding domain in the same polypeptide (*49*, *50*) (**B**). Skp1-based RING E3s include both SCF (Skp1-Cul1/Cdc53-F-box protein) (**C**) and variant Skp1-based complexes (**D**, and see text). SCF and Elongin BC-based E3s include a heterodimeric module composed of a member of the Cullin family (*51*) and the RING finger protein Rbx1 (also referred to as ROC1 or Hrt1) (*4*, *52–55*) that activates ubiquitylation of target proteins by the E2 ubiquitin-conjugating enzymes Cdc34 and Ubc5. SCF complexes include a member of the F-box family of proteins (*56*, *57*), which serve as substrate recognition subunits that bind specifically to and recruit target proteins for ubiquitylation. F-box proteins are linked to a Cul1(Cdc53)/Rbx1 module by the Skp1 adaptor protein, which binds to F-box proteins through a degenerate, ~40 amino acid sequence motif called the F-box. F-box proteins are modular and contain, in addition to an F-box, a protein-protein interaction domain that is responsible for binding selectively to target proteins. The VHL E3 ubiquitin ligase (*5–7*, *58*, *59*) is the founding member of the family of Elongin BC-based E3s (D) (*60*). In the context of the VHL E3, the VHL tumor suppressor protein functions analogously to F-box proteins in the SCF complex to recruit target proteins for ubiquitylation. The VHL protein is linked to a Cul2/Rbx1 module by the ubiquitin-like Elongin B and Skp1-like Elongin C adaptor proteins, which bind to a degenerate, ~12–amino acid BC-box motif present in the VHL and other BC-box proteins (**E**) (*61–63*). Mammalian cells may contain as many as 50 F-box and 50 BC-box proteins, which could all function at least in part as substrate recognition subunits of E3s.

function, and, most recently, regulation of the activity of the yeast transcription factor Met4 (*32*). Met4 binds the promoters of methionine-regulated genes and, in conjunction with other transcription factors, including the basic helix-loop-helix transcription factor Cbf1, activates their transcription in yeast deprived of methionine. Genetic studies had identified an SCF E3 containing the F-box protein Met30 as an inhibitor of Met4-dependent transcription. Recently, Met4 has been shown to be inactivated by SCF^{Met30} in yeast grown in a plentiful supply of methionine by two mechanisms. Rouillon *et al.* showed that, under some conditions, SCF^{Met30} can inactivate Met4 by classic ubiquitin-dependent proteolysis (*33*). Kaiser *et al.* showed that, under other conditions, Met4 can also be inactivated by SCF^{Met30}-dependent ubiquitylation, without proteolysis (*32*). These authors reported that ubiquitylated Met4 is still able to bind to its promoters; however, it fails to interact properly with Cbf1 to reconstitute an active Met4 transcription factor. Notably, in this case, activation of transcription of methionine-repressed genes correlated well with rapid deubiquitylation of Met4, raising the possibility that reversible ubiquitylation of transcription factors, like reversible phosphorylation and acetylation, may turn out to be an important means of regulating their activities.

A role for ubiquitin in transcription activation domain (TAD) function. In a perhaps more unexpected twist on the ubiquitin theme, a variety of evidence suggesting that ubiquitin plays a direct role in transcription activation domain (TAD) function has emerged from several recent studies. Although it was clear that many DNA binding transcription activators are short-lived proteins regulated by the classic ubiquitin-dependent proteolysis pathway (Table 1), Molinari *et al* (*34*) made the intriguing observation that the half-lives in cells of a set of model transcription activators correlated inversely with the potencies of their TADs. By measuring the half-lives and transcription activities of a set of chimeric transcription factors composed of Gal4 DNA binding domains fused to a variety of well-characterized TADs, these authors observed that the transcription factors that activated expression of reporter genes most strongly were ubiquitylated and degraded by the proteasome most rapidly. Notably, they observed that the rapid turnover of their model transcription factors was dependent on the presence of an intact GAL4 DNA binding domain, raising the possibility that transcription factors are most efficiently targeted for ubiquitylation and destruction by the proteasome when they are bound to DNA.

Table 1. Examples of E3 ubiquitin ligases implicated in the regulation of Pol II transcription. References for the information in this table are provided in the supplementary material on *Science* Online at www.sciencemag.org/cgi/content/full/296/5571/1254/DC1.

E3	Class	Target
E6-AP/E6	HECT	p53
Rsp5	HECT	RNA polymerase II large subunit
WWP1	HECT	Lung Kruppel-like factor (LKLF)
Smurf2	HECT	Smad1, Smad2
Mdm2/hdm2	RING	p53 HIF1α and HIF2α?
Siah-1	RING	c-myb
Siah-2	RING	N-CoR
SCF^{Cdc4}	RING, Skp1-based	Gcn4
SCF^{Met30}	RING, Skp1-based	Met4
$SCF^{\beta\text{-}TRCP}$	RING, Skp1-based	IkB, ATF4, β-catenin, Smad3, NF-κB p105
VHL-EloBC-Cul2-Rbx1	RING, Elo BC-based	HIF1α, HIF2α
E4orf6/E1B55K-EloBC-Cul5-Rbx1	RING, Elo BC-based	p53
Med8-EloBC-Cul2-Rbx1	RING, Elo BC-based	?
Siah/SIP/Skp1/Ebi	RING, Skp1-based variant	β-catenin, Tramtrack?
$TAF_{II}250$	Novel	Histone H1

In a related line of research aimed at identifying in transcription factors the destabilization domain that is recognized by the ubiquitin-dependent proteolysis machinery and targets the transcription factor for destruction, Salghetti *et al.* (*35*, *36*) obtained evidence that some acidic TADs are indeed destabilization domains. These authors demonstrated that the well-characterized VP16 acidic TAD functions efficiently as a destabilization domain to target transcription factors containing it for ubiquitylation and destruction by the proteasome (*36*). Highlighting the close relation between acidic TADs and destabilization domains, these authors also demonstrated that destabilization domains present in proteins like *S. cerevisiae* cyclins Cln1 and Cln3, which are not transcription activators, will function as TADs when fused to the Gal4 DNA binding domain.

Recent findings emerging from experiments exploring the role of TADs in regulation of transcription factors by the ubiquitin-dependent proteolysis pathway suggest that TAD-dependent ubiquitylation of transcription factors may not only target them for destruction, but may also be required for their function in transcription activation. The E3 ubiquitin ligases Rsp5/hPRF1 and E6-AP have been reported to serve as coactivators for nuclear receptors (*37*, *38*). In addition, ligand-dependent transcription activation by the estrogen receptor was found to depend on both an active E1 ubiquitin activating enzyme and the proteasome in experiments demonstrating that the estrogen receptor fails to activate transcription of target genes in E1 temperature-sensitive cells grown at the nonpermissive temperature or treated with proteasome inhibitors (*39*).

More direct evidence that TAD-dependent ubiquitylation of transcription factors may be needed to activate them for transcription, however, was obtained by Salghetti *et al.* (*36*) in experiments investigating the mechanism of action of the VP16 TAD. These authors observed that, in *S. cerevisiae* lacking the SCF F-box subunit Met30, the chimeric transcription factor LexA-VP16, which is composed of the LexA DNA binding domain fused to the VP16 TAD, failed not only to be efficiently ubiquitylated and degraded by the proteasome, but also to function as a transcription activator. Thus, VP16-dependent ubiquitylation of LexA-VP16 by the SCF^{Met30} E3 appears to be required not only for its turnover, but also for its transcription activity. Consistent with this interpretation, fusion of a single ubiquitin moiety to LexA-VP16 was sufficient to overcome the requirement for Met30 in transcription, but had no effect on LexA-VP16 half-life. Notably, Met30 does not affect the ability of LexA-VP16 to bind promoters in cells, implying that it may act after the transcription factor has been recruited to the promoter. Taken together, these observations support the notion that ubiquitylation of the LexA-VP16 activator by the SCF^{Met30} ubiquitin ligase is not only a signal for its destruction, but is also required for its function as an activator.

Despite these tantalizing hints that TAD-dependent ubiquitylation of transcription factors may perform dual roles by activating them for transcription, then signaling their demise, transcription factors bearing ubiquitylated TADs have not been shown directly to bind promoters in cells. In addition, not all TADs appear competent to recruit the cellular ubiquitylation machinery. A variety of nonacidic TADs, including the proline-rich CTF and glutamine-rich Sp1 TADs, for example, do not appear to act as destabilization do-

mains (*35*). Nevertheless, taken together, these observations raise the intriguing possibility that some DNA-bound transcription activators may be targeted directly through their TADs for ubiquitylation and activation by E3s and then for destruction by proteasomes closely associated with the Pol II transcription machinery at the promoter. This close linkage between TAD activation and destruction may be imagined to provide an efficient "suicide" mechanism for attenuation of transcriptional signals (Fig. 2). According to this model, the activity of a promoter would depend upon continuous reloading of transcription factors, thereby leading to rapid promoter inactivation upon cessation of signals that lead to transcription factor activation.

Ubiquitin and the Pol II general transcription activation machinery. Although exactly how recruitment of transcription factors to promoters might trigger their ubiquitylation is presently not known, several lines of evidence indicate that (i) signals initiating ubiquitylation of DNA-bound transcription factors could emanate directly from the Pol II general transcription machinery itself, and (ii) in some cases, the E3s responsible for ubiquitylation of transcription proteins could be integral components of the Pol II transcription machinery. It has been known for some time that the *S. cerevisiae* DNA binding transcription factor Gcn4 is tightly regulated by classic ubiquitin-dependent proteolysis by the SCF^{Cdc4} E3, although the signals that trigger Gcn4 ubiquitylation were until very recently poorly understood. Gcn4 controls transcription of a set of genes required for yeast growth in media lacking a suitable nitrogen (N) source. In yeast grown in N-rich media, Gcn4 levels are low, whereas in N-deprived yeast, Gcn4 ubiquitylation is inhibited, and Gcn4 is free to activate its transcriptional program.

Phosphorylation of Gcn4 by the cyclin-dependent kinases Pho85 (*40*) or Srb10 (*41*) is sufficient to signal Gcn4 ubiquitylation by SCF^{Cdc4}. Notably, Srb10 is a subunit of the >1 MD, multiprotein *S. cerevisiae* Mediator complex, which functions in tight association with Pol II and the general transcription factors to promote activation of transcription by Gcn4. From genetic studies, Srb10 was known to be involved in ensuring that expression of Gcn4-dependent genes is "turned off" in yeast grown in N-rich media. Although it is presently not clear whether Srb10, as a subunit of the Mediator, can phosphorylate DNA-bound Gcn4, it is tempting to speculate that, in yeast grown in an N-rich source, any Gcn4 that inappropriately binds to promoters is rapidly phosphorylated by the Srb10 subunit of the adjacent Mediator complex, allowing it to be ubiquitylated by the SCF^{Cdc4} complex and degraded by the proteasome.

Additional evidence for an intimate association of the cellular ubiquitylation machinery and the Pol II general general transcription activation machinery has come with the finding that a component of the mammalian Mediator complex, mMED8 (also known as ARC32), is an Elongin BC-box protein that can assemble with Elongins B and C, Cul2, and Rbx1 to form an E3 related to the VHL E3 (*42*). The E3 subunits Cul2, Elongins B and C, and Rbx1 were found to cofractionate with mMED8 and other Mediator subunits as components of a >1-MD complex with associated ubiquitin ligase activity. Although substrates for the mMED8 E3 have not yet been identified, these findings raise the possibility that one function of mMED8 may be to recruit ubiquitin ligase activity directly to the Mediator, where it could target transcription activators, other Mediator subunits, or Pol II and the general initiation factors.

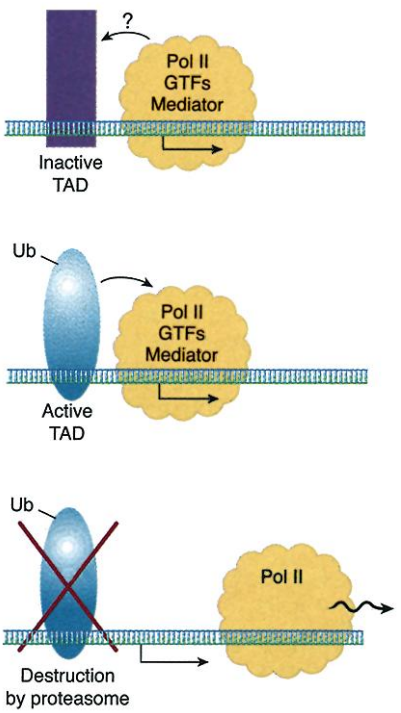

Fig. 2. A "suicide" model for concerted TAD activation and destruction. Based on the model of Salghetti *et al* (*36*), binding of some transcription factors to promoters triggers their ubiquitylation, which simultaneously activates them to initiate a round of transcription and tags them for subsequent destruction by the proteasome in a step that attenuates transcription. Although the precise signal(s) responsible for this process remains unclear, it may in some cases emanate from Mediator, as suggested by the observations (i) that the Srb10 kinase subunit of Mediator can signal ubiquitylation and destruction of the transcriptional activator Gcn4 and (ii) that the MED8 subunit of Mediator can assemble into an Elongin BC-based E3.

Finally, the $TAF_{II}250$ subunit of Pol II general initiation factor TFIID has recently been shown to possess the ability to monoubiquitylate histone H1 in vitro (*43*). $TAF_{II}250$ represents an interesting variation on the classic ubiquitylation enzymes, because it has both E1 ubiquitin-activating and E2 ubiquitin-conjugating activities. That histone H1 may be a bona fide target in cells for $TAF_{II}250$-dependent ubiquitylation is suggested by the finding that $TAF_{II}250$ point mutations that block ubiquitylation of histone H1 in vitro also lead to decreases in the accumulation of ubiquitylated H1 in *Drosophila* embryos. In addition, these same $TAF_{II}250$ mutations result in defects in activation of genes controlled by the transcriptional factor Dorsal, but not in a general transcription defect. Although it is not yet known whether histone H1 is the sole target of $TAF_{II}250$, it is tempting to speculate that $TAF_{II}250$ could also ubiquitylate and regulate the activities of other components of the Pol II transcription machinery, because of its close proximity to these transcription proteins in the Pol II initiation complex.

Proteasomal Subunits as Transcription Regulators

Although this review has focused on emerging roles of ubiquitin in the regulation of Pol II transcription, it is noteworthy that recent findings have also implicated components of ubiquitin's close colleague, the proteasome, in Pol II transcription. In an elegant series of experiments Johnston, Kodadek, and co-workers have obtained evidence that a subcomplex of the 19*S* regulatory particle of the proteasome participates in transcriptional regulation. The 19*S* particle includes several AAA ATPases (ATPases associated with a variety of cellular activities), which function at least in part to promote ATP-dependent unfolding of proteins before their degradation in the catalytic 20*S* proteasome core. Genetic studies had suggested a role for the 19*S* AAA ATPase subunit Sug1 in transcription activation, but they had not ruled out the possibility that Sug1 participates only indirectly in this process. Recently, Ferdous *et al.* (*44*) found that the 19*S* particle of the proteasome is capable of potently activating Pol II transcription elongation in vitro by a mechanism that is independent of proteolysis. Further supporting an important role for components of the 19*S* particle in transcription, Gonzales *et al.* (*45*) obtained evidence that a 19*S* subcomplex that includes the AAA ATPase subunits, but not several other 19*S* subunits needed for proteasome function, is recruited directly to the promoter and transcribed regions of some genes by the transcription activator Gal4. Although the precise mechanism(s) by which the 19*S* AAA ATPases function in Pol II transcription is presently un-

known, it is tempting to speculate that they may regulate transcription by promoting ATP-dependent protein unfolding (or partial unfolding), allowing restructuring of transcriptional regulatory complexes in a way that results in activation of transcription initiation and/or elongation.

Prospects for the Future

New roles for ubiquitin in regulation of Pol II transcription are being discovered at an accelerating pace. Ongoing studies are revealing not only the integral role that ubiquitin plays in Pol II transcription, but also the complex and multifaceted nature of ubiquitin's participation in this process. Future efforts to understand in detail the many roles that ubiquitin plays in transcription will undoubtedly turn up additional, previously unknown E3s, their protein targets, and their mechanisms of action in regulating eukaryotic mRNA synthesis. In addition, future investigations of ubiquitin's role in TAD activation and destruction can be expected to reveal a fundamental and perhaps "general" role for ubiquitin in some of the most basic aspects of transcription activation and repression. Finally, evidence that components of the multisubunit proteasome have direct roles in Pol II transcription has opened up new and intriguing avenues of research on this complex macromolecular machine.

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