

# Molecular Biology: What Ubiquitin Can Do for Transcription Dispatch

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**Ubiquitin, the peptide ‘tag’ that targets eukaryotic proteins for degradation by the proteasome, has also been implicated in transcriptional activation. The mechanism of gene activation might include recruitment of a transcriptional elongation factor by ubiquitinated activators.**

Transcriptional activation in eukaryotes is a highly regulated process requiring the concerted interactions of DNA-binding transcriptional activators, general transcription factors and coactivator proteins to stimulate the recruitment or activity of RNA polymerase II at the appropriate gene promoters in response to biological signals. Activators are often unstable proteins, which may be one way to maintain tight control over their function. Ubiquitination of activators can lead to their destruction by the proteasome, but might also function in the transcriptional activation event itself [1,2]. A recent report in *Current Biology* by Kurosu and Peterlin [3] offers an explicit mechanism for the role of ubiquitin in the stimulation of transcriptional elongation.

Some transcriptional activators stimulate transcriptional initiation by recruiting general transcription factors and RNA polymerase II into a preinitiation complex. Other activators foster the release of RNA polymerase II from the promoter, or boost the elongation rate through the transcribed gene [4]. For example, the activation domains of the HIV Tat and the herpesvirus VP16 proteins can interact with P-TEFb, a protein complex required for the suppression of pausing of RNA polymerase II [5]. P-TEFb comprises a C-type cyclin (most often CycT1) and a cyclin-dependent kinase (Cdk9) [5], and can phosphorylate the carboxy-terminal domain (CTD) of the largest subunit of RNA polymerase II and also certain negative elongation factors. These phosphorylation events allow efficient elongation and also help recruit the RNA processing proteins that travel with RNA polymerase II for cotranslational processing of the nascent mRNA [6,7].

Transcription activators are tightly regulated through diverse mechanisms that control subcellular localization, protein stability and protein activity, often mediated by post-translational modifications such as phosphorylation, acetylation, glycosylation and ubiquitination. Ubiquitin is a polypeptide of 76 amino acids that typically gets covalently attached to proteins destined for destruction by the proteasome (reviewed in [8,9]). Many transcriptional activators are unstable proteins degraded by the ubiquitin–proteasome pathway [1,10]. This instability is often used to restrain

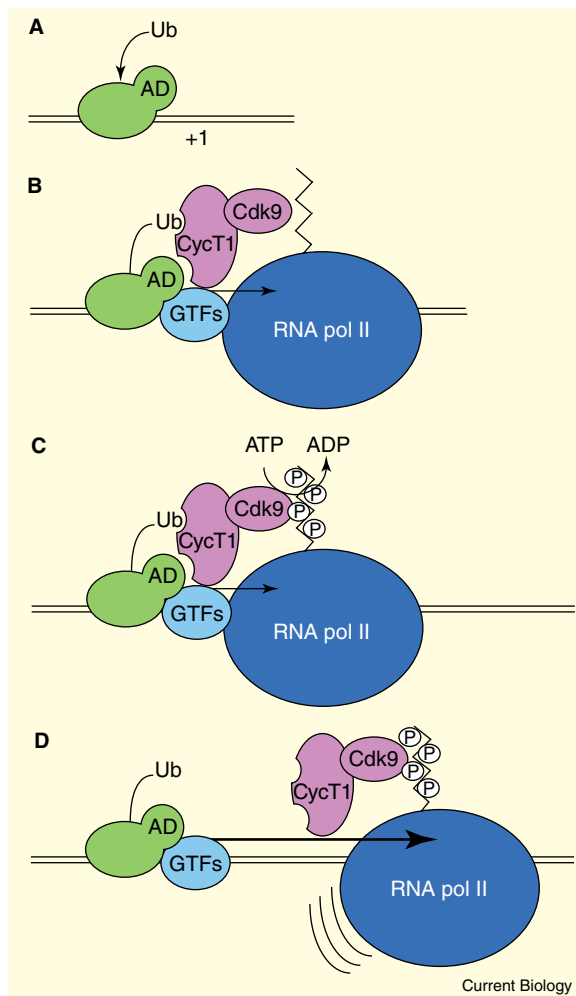
the activity of an activator at times when its target genes should not be expressed, or to quench its activity so that the transcriptional response to a stimulus can be quickly dampened. Interestingly, the activation domains of many regulatory proteins overlap with the signals responsible for the degradation [2,10,11], leading to the suggestion that destruction of the activator might be a requisite step in the mechanism of transcriptional activation [1].

Additional evidence suggests that the proteasome itself can be intimately involved in transcriptional activation. The yeast Sug1 protein, a component of the 19S subcomplex of the proteasome, exhibits coactivator properties in the activation of galactose regulated genes [12]. Proteasome components have been detected at actively transcribed genes using chromatin immunoprecipitation assays [13,14]. Turnover of the estrogen receptor by the proteasome is important for estrogen-regulated transcription [14,15]. Some genetic experiments implicate the proteasome specifically in transcriptional elongation [16]. The proteasome (or parts thereof) may help reorganize or disassemble the preinitiation complex, freeing RNA polymerase II to progress into elongation. In this context, the proteasome might not be executing its proteolytic function [17], but might instead use its ATPases as chaperones for remodeling protein conformations or interactions.

Indeed, ubiquitination does not always presage destruction. Non-degradative functions of ubiquitin have emerged in recent years, including regulation of protein location, protein function, and protein–protein interactions [18]. Several reports suggest that such nonproteolytic functions are important for the activity of transcriptional activation domains from the VP16 and Tat proteins [19,20]. In these studies, the function of a given activator protein was diminished in cells lacking the appropriate ubiquitin ligase — which attaches ubiquitin to specific target proteins — but the transcriptional function was restored by genetically fusing the ubiquitin polypeptide to the activator protein.

Kurosu and Peterlin [3] report evidence suggesting an explicit mechanism by which ubiquitination of activators might facilitate transcriptional elongation. They constructed chimeric genes joining the activation domain of VP16 — wild-type or mutant versions — with either the LexA DNA-binding domain (LexA–VP16) or the RNA-binding domain of the HIV Rev protein (Rev–VP16). When the fusion proteins bearing the wild-type VP16 activation domain were expressed in mammalian cells, they were ubiquitinated and they stimulated expression of cognate reporter genes.

Three aspects of these experiments suggest that transcriptional elongation was boosted. First, the activation was observed using either a DNA-based tether (LexA binding to a promoter sequence) or an RNA-based tether (Rev binding to an initial portion of the reporter gene transcript). Second, the stimulation was blocked by a dominant-negative form of the Cdk9



**Figure 1. Ubiquitination and the control of transcription.** (A) Ubiquitination of an activator protein can be triggered by an acidic activation domain (AD). (B) The ubiquitin moiety and the AD itself can bind simultaneously to distinct regions of the CycT1 component of P-TEFb. (C) The Cdk9 subunit of P-TEFb then phosphorylates Ser residues in the carboxy-terminal tail of the large subunit of RNA polymerase II, resulting in (D) enhanced elongation efficiency and recruitment of RNA-processing enzymes.

protein, indicating that the transcriptional activation depended on P-TEFb. And third, use of the mutant VP16 domain resulted in less abundant expression of distal regions of the reporter gene relative to more proximal segments, suggesting pausing or premature termination of transcription.

The real news derives from additional experiments that point quite directly to a role for ubiquitin in this transcriptional elongation activity. When ubiquitin was artificially fused to LexA-VP16 — generating ubiquitin-LexA-VP16 — expression of the reporter gene was augmented and the deleterious effect of the VP16 F442A mutation was suppressed. LexA-VP16 was shown to bind to the CycT1 subunit of P-TEFb *in vitro*, and ubiquitin-LexA-VP16 bound even more avidly to CycT1. In fact, VP16 and ubiquitin bound to different regions of CycT1, so when both are present

the interaction is stronger. The model that emerges is that the presence of ubiquitin attached to an activator protein results in enhanced recruitment of P-TEFb and thus enhanced transcriptional elongation (Figure 1).

One caveat for these experiments arises from the use of highly artificial fusion proteins and reporter genes, and so it will be important to test this hypothesis in more native biological contexts. Nonetheless, the strength of the new report by Kurosu and Peterlin [3] is their identification of a mechanistic and testable hypothesis for the function of the ubiquitin that gets attached to transcriptional activator proteins. In showing that this ubiquitin augments binding of the P-TEFb elongation factor and enhances synthesis of full-length transcripts, this model connects the ubiquitination of acidic activators with the ability to stimulate transcriptional elongation. This will not be the only mechanism for transcriptional activation; other classes of activators with glutamine-rich or proline-rich activation domains are not ubiquitinated [10] and do not stimulate transcriptional elongation [4]. And it also may not be the only role that ubiquitin plays in transcriptional activation; other mechanisms, particularly those that invoke the proteasome as an active participant, may also emerge. But the biochemical hypothesis proposed by Kurosu and Peterlin [3] provides an interesting focus for future experiments.

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