

belemnite (PDB) standard], were injected via the GC-C III interface to the IRMS for the computation of $\delta^{13}\text{C}$ values of sample compounds. A set of standard *n*-alkanes with known $\delta^{13}\text{C}$ values acquired from Indiana University were measured before sample analyses to ensure accuracy of the $\delta^{13}\text{C}$ measurement. The standard deviation for duplicate analyses is $< \pm 0.3\%$. $\delta^{13}\text{C}$ TOC was determined by combusting decarbonated (with 10% HCl) sediments in an elemental analyzer and subsequently collecting and cryogenically purifying the evolved CO_2 . The CO_2 samples were then measured on dual-inlet IRMS for isotopic ratios. For Alta Babicora sediments, a cold finger was used to further concentrate the CO_2 for measurement because of the exceptionally low organic content (0.2 to 0.6%). Carbon isotope values are expressed relative to the V-PDB standard.

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31. We thank T. Webb III and S. C. Clemens for insightful comments, M. F. Rosenmeier and J. H. Curtis for kind assistance during subsampling of Quexil sediment cores, B. W. Leyden for supplying Quexil pollen data, H. Fischer for supplying CO_2 data from the Taylor Dome ice core, and B. D. Walizer for assistance during $\delta^{13}\text{C}$ TOC analyses. Supported by grants from NSF (EAR-9814584 and ATM-0081478) and the American Chemical Society–Petroleum Research Fund (35208-G2).

23 February 2001; accepted 31 July 2001

Regulation of Transcriptional Activation Domain Function by Ubiquitin

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The ability of transcriptional activation domains (TADs) to signal ubiquitin-mediated proteolysis suggests an involvement of the ubiquitin-proteasome pathway in transcription. To probe this involvement, we asked how ubiquitylation regulates the activity of a transcription factor containing the VP16 TAD. We show that the VP16 TAD signals ubiquitylation through the Met30 ubiquitin-ligase and that Met30 is also required for the VP16 TAD to activate transcription. The requirement for Met30 in transcription is circumvented by fusion of ubiquitin to the VP16 activator, demonstrating that activator ubiquitylation is essential for transcriptional activation. We propose that ubiquitylation regulates TAD function by serving as a dual signal for activation and activator destruction.

Many transcription factors are unstable proteins that are destroyed by ubiquitin (Ub)-mediated proteolysis (1), a process in which covalent attachment of Ub to proteins signals their destruction by the proteasome (2). In most transcription factors, the domain that signals their ubiquitylation—the “degron”—overlaps closely with a transcriptional activation domain (TAD). Indeed, this overlap is both widespread and intimate: Mutational analysis of TADs (3–5) reveals a close correlation between transcriptional activation and proteolysis. The unexpected convergence of transcription and proteolytic signaling elements raises the possibility that the Ub-proteasome pathway is involved in transcription. We tested this hypothesis by examining the role that the ubiquitylation machinery

plays in transcriptional activation by the VP16 activation domain (6).

Substrate targeting by the ubiquitylation machinery is carried out by Ub-ligases (2), which interact with the degron and recruit Ub-conjugating enzymes to the substrate protein. Because of the key role of Ub-ligases in substrate recognition, we sought to identify the Ub-ligase that targets the VP16 TAD in *Saccharomyces cerevisiae*. We fused the VP16 TAD to the bacterial DNA binding protein LexA (7) and expressed the fusion protein in yeast. For comparison, we also fused LexA to the TAD-degrons from Myc (3) and from the yeast cyclin Cln3 (5). Pulse-chase analysis (Fig. 1A, lanes 1 through 4) revealed that all three TADs acted as degrons in this setting, destabilizing the LexA protein. To identify the Ub-ligase for VP16, we next examined the stability of LexA-VP16 in yeast strains defective for various components of the Ub-proteasome pathway, including Ubc2, Cdc4, Met30, and Grr1. This analysis revealed that LexA-VP16 was stabilized by loss of Met30 (Fig. 1A), a substrate-recognition component of the SCF Ub-ligase family (8). The dependence on

Met30 was specific to LexA-VP16 because deletion of *Met30* had little effect on the stabilities of LexA-Myc and LexA-Cln3 (Fig. 1A). Consistent with a specific role for Met30 in VP16 degron function, Met30 associated with LexA-VP16—and not other LexA-fusion proteins—in vitro (Fig. 1B, compare lanes 2 and 4 with lane 6), and Met30 was required for LexA-VP16 ubiquitylation in vivo (Fig. 1C, compare lanes 3 and 6) (9). Taken together, these data demonstrate that the Met30 Ub-ligase is specifically required for degron function of the VP16 TAD.

We next examined whether loss of Met30 affected transcriptional activation by VP16 (Fig. 2). A modified GAL1 promoter carrying two LexA binding sites and driving expression of β -galactosidase (10), was integrated into yeast strains that either contained or lacked a functional *Met30* locus. We then measured the ability of each LexA fusion protein to activate reporter gene expression in these cells (Fig. 2A, transcription). As expected, the Myc, Cln3, and VP16 TADs potently activated reporter gene expression in the presence of Met30. However, in the absence of Met30, the VP16 TAD failed to activate β -galactosidase expression. As observed with proteolysis (Fig. 1A), the effect of loss of Met30 was specific to VP16 because transcriptional activation by LexA-Myc and LexA-Cln3 remained constant. Thus, although the LexA-VP16 activator is more stable and accumulates to twofold higher levels (Fig. 2A, protein) in *Met30*-null cells, it is unable to activate transcription in the absence of Met30 (11). The specific loss of VP16 transcriptional activity reveals that Met30 plays an essential role in both the TAD and degron function of the VP16 activation domain.

To determine whether loss of Met30 attenuates VP16 activity through an indirect mechanism, we asked whether LexA-VP16 displays any activity in *Met30*-null cells. Chromatin immunoprecipitation (ChIP) analysis (Fig. 2B) revealed that LexA-VP16 efficiently interacts with promoter DNA in the absence of Met30 in vivo (compare lanes 3 and 6), demonstrating that there is not a global defect in the folding or

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location of the LexA-VP16 protein. Moreover, the VP16 TAD itself retained at least one function in *Met30*-null yeast, the ability to stimulate

DNA replication (Fig. 2C). Many TADs can stimulate DNA replication (12), perhaps through their ability to direct chromatin remodeling. Using a plasmid stability assay in which the B3 element of the ARS1 replication origin (13) is replaced by a single LexA binding site, we found that LexA-VP16 stimulated DNA replication equally well in the presence or absence of *Met30*, as did all LexA-TAD fusions. The selective retention of this function demonstrates that VP16 TAD activity is not universally blocked in *Met30*-null yeast and strongly supports the concept that the role of *Met30* in VP16 TAD function is specifi-

cally related to transcriptional activation.

We next probed the role of *Met30* in transcriptional activation by the VP16 TAD. We speculated that *Met30*-mediated ubiquitylation of LexA-VP16 may be essential for transcriptional activation. If this was the case, it might be possible to circumvent the requirement for *Met30* by directly ubiquitylating the LexA-VP16 protein. To test this hypothesis, we fused a single nonremovable Ub to the amino terminus of LexA-VP16 (Ub-VP16) and again assayed protein stability (Fig. 3A) and transcriptional activation (Fig. 3B). Although direct fusion to Ub did not

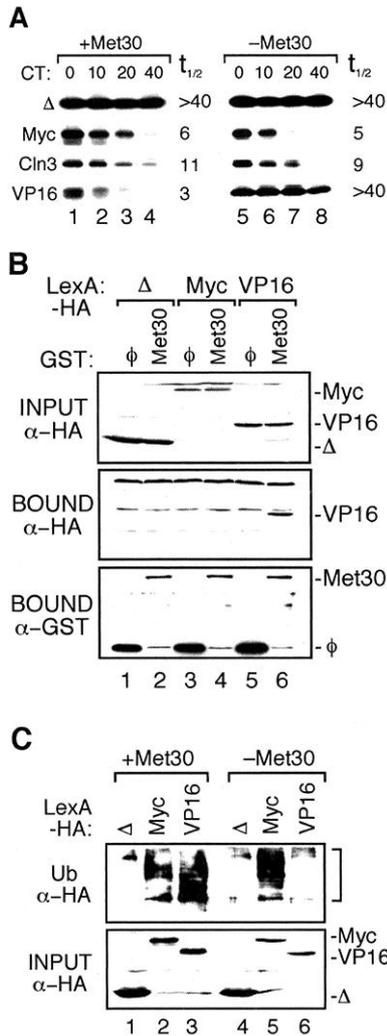


Fig. 1. *Met30* is required for degron function of the VP16 TAD. (A) Stability of LexA fusion activators. Pulse-chase analysis was used to directly measure the stability of LexA alone (Δ), or the indicated fusion protein in *Met30* (+) or (-) yeast (20). Shown are the radiolabeled HA-tagged proteins present in the initial pulse (0) and at each time point in the chase. Because deletion of *Met30* is lethal unless *Met4* is first deleted (17), each yeast strain also lacked a functional *Met4* locus. CT, chase-time, in minutes; $t_{1/2}$, protein half-life, in minutes. (B) The VP16 TAD associates with *Met30* in vitro. A GST pull-down (21) was used to examine association of HA-tagged LexA-fusions with either GST (ϕ) or GST-*Met30*. Shown are LexA proteins in the input (INPUT α -HA) and bound to glutathione agarose (BOUND α -HA), and GST fusion proteins bound to glutathione agarose (BOUND α -GST). (C) *Met30* is required for the VP16 TAD to signal ubiquitylation. HA-tagged LexA proteins (INPUT α -HA) were isolated from yeast expressing polyhistidine-tagged Ub (22), ubiquitylated proteins recovered by nickel affinity chromatography, and HA-tagged proteins detected by immunoblotting (Ub α -HA).

Fig. 2. *Met30* is required for transcriptional activity of the VP16 TAD. (A) Transcriptional activation by LexA-fusion proteins. LexA-fusion proteins were expressed in *Met30* (+) or (-) yeast and transcription assayed by measuring β -galactosidase expression (Miller units) from an integrated LexA- β -galactosidase reporter. The bottom panel labeled "Protein" shows steady-state levels of the LexA fusion proteins as determined by immunoblotting. LexA-VP16 bands are bracketed (23). (B) In vivo promoter occupancy by LexA fusion proteins. The indicated yeast were subject to ChIP analysis (24). PCR was used to quantitate association of each LexA fusion protein with a fragment corresponding to the integrated LexA reporter ("1"), or the transcriptionally silent GAL1 promoter that lacked LexA binding sites ("2"). Lane 7 shows ChIP analysis from yeast that do not express a LexA-fusion protein. Lane 8 shows the PCR profile of a DNA sample that was not subject to immunoprecipitation. (C) LexA-VP16 stimulates DNA replication in the absence of *Met30*. A plasmid stability assay (25) was used to measure the ability of the indicated LexA fusion proteins to drive maintenance of a reporter plasmid containing a *URA3* selectable marker. Plasmid stability is expressed as a fraction of yeast colonies that retain the *URA3* marker after growth under nonselective conditions.

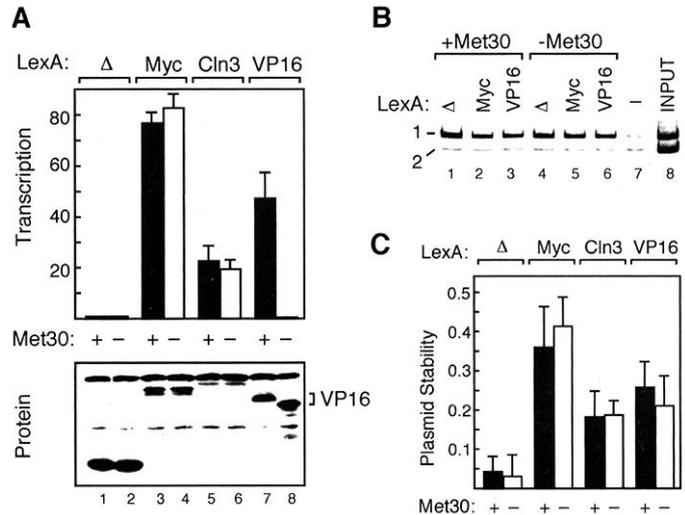
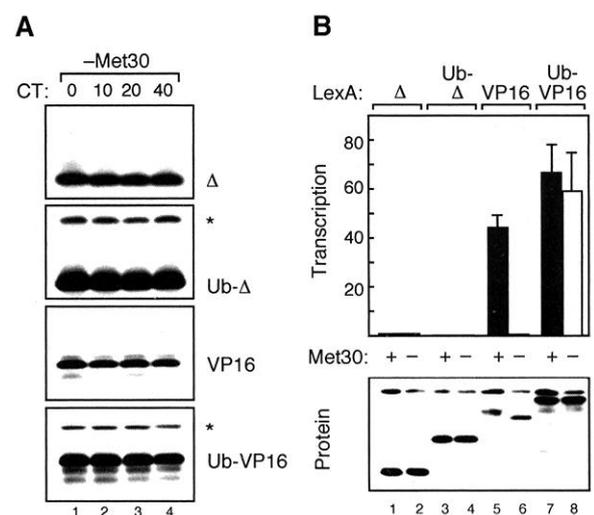


Fig. 3. Direct fusion to Ub allows LexA-VP16 to activate transcription in the absence of *Met30*. (A) Linear fusion to Ub does not restore LexA-VP16 turnover in *Met30*-null yeast. Ub was fused in-frame to the amino terminus of LexA alone (Ub- Δ) or to LexA-VP16 (Ub-VP16) (26). Pulse-chase analysis was used to measure the stability of these proteins in *Met30*-null yeast. The linear fusion to Ub resulted in the formation of an additional protein species (denoted by asterisk) that is probably due to monoubiquitylation of the fusion protein (19). (B) Linear fusion to Ub restores transcriptional activation by LexA-VP16 in *Met30*-null yeast. Transcription and protein levels for each LexA-fusion protein were measured as described in Fig. 2.



restore LexA-VP16 destruction in *Met30*-null cells (Fig. 3A), the fusion did rescue transcriptional activation, restoring wild-type levels of VP16 TAD activity (Fig. 3B, compare VP16 and Ub-VP16 in the *Met30*-null cells). Moreover, both Ub and the VP16 TAD are required for transcriptional activation in the *Met30*-null cells because Ub alone fused to LexA (Ub-Δ) did not activate transcription (14). The observation that *Met30*'s role in transcription can be complemented by fusion of LexA-VP16 to Ub argues that *Met30* co-activates the VP16 TAD by signaling LexA-VP16 ubiquitylation. Moreover, the metabolic stability of the Ub-VP16 protein (Fig. 3A) demonstrates that it is *Met30*-mediated ubiquitylation, not destruction, that is required for transcriptional activation.

The requirement of ubiquitylation for VP16 activator function reveals that the degron function of the VP16 TAD is intimately tied to its ability to activate transcription. The link between these processes provides a simple explanation for the frequent and intimate overlap of TADs and degrons (3–5). This requirement for ubiquitylation, which has not been observed in vitro, reveals a function for Ub distinct from its role in proteolysis (2). Recent evidence has demonstrated that the 19S subunit of the proteasome plays an essential role in transcriptional elongation (15). Given the role of the 19S complex as a Ub binding module (16), it is possible that activator ubiquitylation serves to recruit the 19S complex to promoters, where the chaperone functions of this complex promote transcription elongation.

Although our data demonstrate that proteolysis is not required for transcriptional activation, it is important to note that *Met30* does direct LexA-VP16 destruction. This suggests that activator destruction by the proteasome is a natural consequence of ubiquitylation. Because of the dual role of Ub in transcriptional activation and activator destruction, therefore, we propose that Ub “licenses” transcription factors by linking their activity to their destruction. We imagine that non-ubiquitylated activators are stable and inactive. Interactions of an activator with a Ub-ligase result in activator ubiquitylation, which simultaneously activates the transcription factor and primes it for destruction by the proteasome. Given the large number of transcription factors that are targeted for Ub-mediated proteolysis, it is possible that many transcription factors are regulated through this mechanism.

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21. LexA fusion proteins were expressed in CC849-1B, along with either glutathione S-transferase (GST) or GST-*Met30* (18) under the control of the GAL1 promoter. Log-phase cultures were induced with galactose, proteins were harvested, and GST proteins were collected on glutathione agarose (18).
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26. Ub coding sequences were polymerase chain reaction (PCR)-amplified from yeast genomic DNA with two modifications: (i) the carboxy-terminal glycine residue was changed to alanine to prevent removal of the Ub moiety by isopeptidases (19), and (ii) the T7-epitope tag sequence was added at the carboxy terminus of Ub.
27. For reagents, we thank D. Finley, W. Herr, R. Li, M. Ptashne, and D. Thomas. We thank S. Grewal, R. Li, A. Matapurkar, and V. Valmееkam for technical help. A.A.C. is a George A. and Marjorie H. Anderson Fellow of the Watson School of Biological Sciences and a Howard Hughes Medical Institute Predoctoral Fellow. W.P.T. is a Kimmel Scholar. Supported by the Cold Spring Harbor Laboratory Cancer Center Support Grant CA45508 and by USPHS grant CA-13106 from the National Cancer Institute.

30 April 2001; accepted 2 July 2001
 Published online 19 July 2001;
 10.1126/science.1062079
 Include this information when citing this paper.

Duration of Nuclear NF-κB Action Regulated by Reversible Acetylation

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The nuclear expression and action of the nuclear factor kappa B (NF-κB) transcription factor requires signal-coupled phosphorylation and degradation of the IκB inhibitors, which normally bind and sequester this pleiotropically active factor in the cytoplasm. The subsequent molecular events that regulate the termination of nuclear NF-κB action remain poorly defined, although the activation of de novo IκBα gene expression by NF-κB likely plays a key role. Our studies now demonstrate that the RelA subunit of NF-κB is subject to inducible acetylation and that acetylated forms of RelA interact weakly, if at all, with IκBα. Acetylated RelA is subsequently deacetylated through a specific interaction with histone deacetylase 3 (HDAC3). This deacetylation reaction promotes effective binding to IκBα and leads in turn to IκBα-dependent nuclear export of the complex through a chromosomal region maintenance-1 (CRM-1)-dependent pathway. Deacetylation of RelA by HDAC3 thus acts as an intranuclear molecular switch that both controls the duration of the NF-κB transcriptional response and contributes to the replenishment of the depleted cytoplasmic pool of latent NF-κB-IκBα complexes.

NF-κB corresponds to an inducible transcription factor complex that plays a pivotal role in regulating the inflammatory, immune, and anti-apoptotic responses in mammals (1, 2). The prototypical NF-κB complex, which corresponds to a heterodimer of p50 and RelA

subunits, is sequestered in the cytoplasm by its assembly with a family of inhibitory proteins termed the IκBs (1). Stimulus-induced phosphorylation of two NH₂-terminal serines in the IκBs, mediated by a macromolecular IκB kinase complex (IKK) (3), triggers the