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The F Box Protein Dsg1/Mdm30 Is a Transcriptional Coactivator that Stimulates Gal4 Turnover and Cotranscriptional mRNA Processing

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Summary

We report here that the prototypical yeast transcription factor Gal4 undergoes two distinct modes of ubiquitin-mediated proteolysis: one that occurs independent of transcription and restricts Gal4 function, and another that is transcription coupled and essential for productive activation of Gal4 target genes. Destruction of transcriptionally active Gal4 depends on an F box protein called Dsg1/Mdm30. In the absence of Dsg1, Gal4 is stable, nonubiquitylated, and unable to productively stimulate transcription. Analysis of the phenotype of *dsg1*-null yeast reveals a striking disconnect between *GAL* gene RNA and protein levels; in the absence of Dsg1, Gal4 target genes are transcribed, but the resulting RNAs are not translated. The translational defects of these RNAs are related to defects in phosphorylation of the RNA polymerase II carboxy-terminal domain, which in turn affects recruitment of RNA processing machinery. We propose that Gal4 ubiquitylation and destruction are required for initiation-competent transcription complexes to transition to fully mature elongating complexes capable of appropriate mRNA processing.

Introduction

Transcription in eukaryotes is a highly controlled process that involves the concerted interaction of DNA bound activators, components of the general transcriptional machinery, and chromatin (Kadonaga, 2004). Production of mRNAs requires that RNA polymerase II (pol II) not only be recruited to target genes when appropriate, but that its activity is coordinated with events required for pre-mRNA processing, such as capping, splicing, and polyadenylation (Maniatis and Reed, 2002). To coordinate the diverse aspects of the transcription process, a variety of strategies—many of which depend on posttranslational modification of transcription factors—have evolved. Perhaps one of the most interesting and least understood modifications used to regulate transcription is ubiquitylation (reviewed in Lipford and Deshaies, 2003; Muratani and Tansey, 2003).

Ubiquitin (Ub) is a highly conserved 76 amino-acid protein that is covalently linked to substrates via an enzymatic cascade, the last stage of which is mediated by a so-called Ub ligase. The covalent attachment of Ub to proteins usually results in their rapid destruction by the proteasome, although nonproteolytic functions of Ub (Terrell et al., 1998), and of the proteasome (Russell et al., 1999), have been described. Evidence suggests that components of the Ub proteasome system regulate transcription through both proteolytic and nonproteolytic activities (Muratani and Tansey, 2003). For example, destruction of transcriptional regulators can promote coactivator exchange on promoter sites in vivo (Ostendorff et al., 2002), whereas monoubiquitylation of histone H2B—which is not associated with proteolysis—regulates histone H3 methylation and transcriptional gene silencing (Sun and Allis, 2002).

We are interested in how transcriptional activators are regulated by ubiquitylation. Four lines of evidence suggest that the ability of activators to engage the Ub system is connected to transcription. First, transcriptional activation domains (TADs) overlap with proteolytic signaling elements (degrons) in the majority of transcription factors characterized (Salghetti et al., 2000). Second, acidic-type TADs function as potent degrons, and their ability to signal protein turnover correlates with their ability to activate transcription (Molinari et al., 1999; Salghetti et al., 2000). Third, the stability of numerous transcription factors, such as the retinoic acid receptor (Bastien and Rochette-Egly, 2004), *decreases* when these factors are active. And fourth, we have shown that the Ub ligase Met30 is essential for both the destruction and activity of a chimeric transcription factor (Salghetti et al., 2001), suggesting that activator ubiquitylation and turnover is tightly connected to transcription—and possibly required for normal levels of activation.

Although the concept that activator ubiquitylation and turnover are coupled to transcription can explain the overlap of TADs and degrons, as well as the growing number of Ub ligases that are transcriptional coactivators (Muratani and Tansey, 2003), several important issues are unresolved. One issue is how to reconcile the above observations with examples where transcriptional activation and activator turnover are clearly uncoupled, such as in the Wnt signaling pathway, where destruction of β-catenin antagonizes its function (Aberle et al., 1997). Another issue is whether a mechanistic connection between activity and proteolysis exists for a native transcription factor. Finally, perhaps most importantly, is to identify the step in transcription that is linked to activator destruction. Only when this step is identified can the full significance of activator destruction be understood.

To address these issues, we have studied turnover of the yeast activator Gal4. We find that there are two distinct modes of Gal4 destruction by the Ub system: one that is separate from transcription and restricts *GAL* gene activation, and another that is tightly coupled to transcription and required for productive mRNA synthesis. The transcription-coupled destruction of Gal4 is associated with post-initiation changes in phosphorylation of the pol II carboxy-terminal domain (CTD) and

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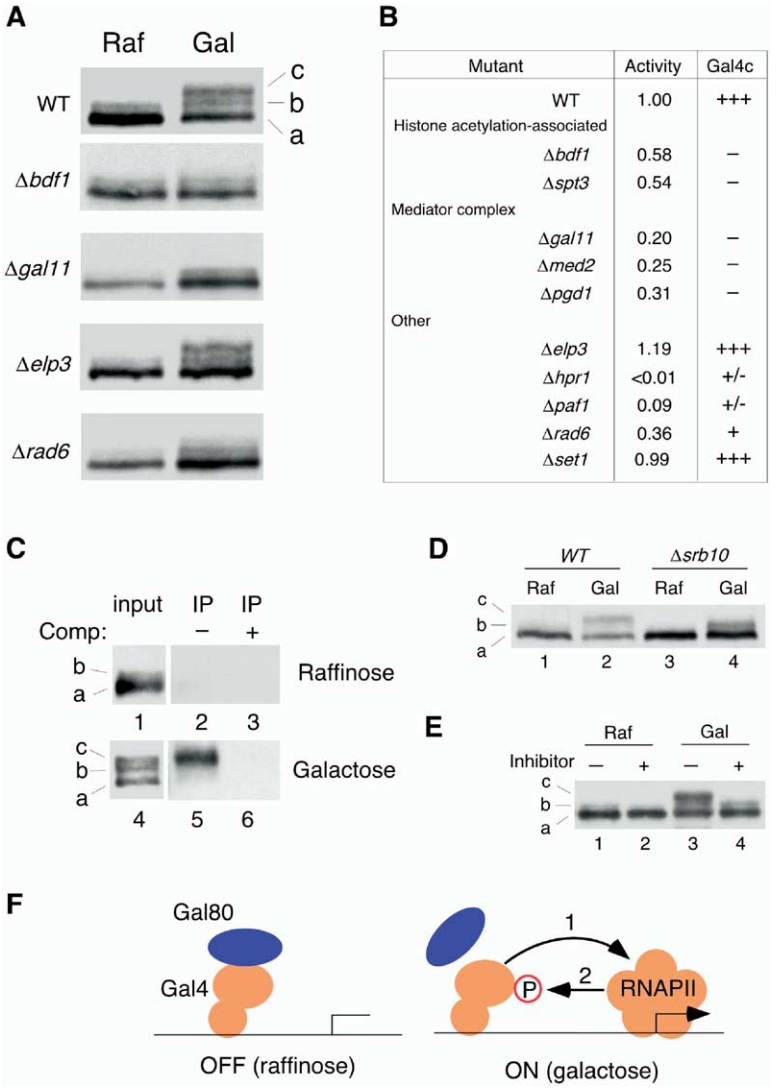

Figure 1. Transcription-Associated Phosphorylation of Gal4

(A) Mutations in the transcriptional machinery affect Gal4c formation. Protein extracts were prepared from wild-type (WT) yeast JT1 (Table S1) and congenic deletion strains expressing HA-tagged Gal4 (p2HG4), grown in either non-inducing (Raf) or inducing (Gal) conditions. Western blotting (WB; Ab12CA5) indicates the presence of three isoforms in galactose-containing media, labeled "a, b, c."

(B) Gal4c formation correlates with *GAL* reporter gene activity. Comparison of the effects of the indicated gene deletions on *GAL1-LacZ* (β-galactosidase) activity versus Gal4c steady-state levels in galactose-containing media is shown.

(C) Ser699 phosphorylation is Gal4c specific. Total proteins (lanes 1 and 4) from WT yeast expressing HA-tagged Gal4 (JT1+p2HG4) were sequentially immunoprecipitated with an anti-Gal4 DBD antibody, followed by an anti-phospho S699 antibody. Where indicated, specific competitor phosphopeptide was included in the second IP reaction. Immunoprecipitated proteins were visualized by WB with the anti-HA antibody 12CA5.

(D) Isoform c formation requires Srb10.

(E) Isoform c formation requires Kin28. Yeast kin28-as, expressing HA-tagged Gal4, were treated with DMSO (lanes 1 and 3) or inhibitor (1-NA: lanes 2 and 4). WB (Ab12CA5) was used to visualize Gal4.

(F) Model for Gal4c formation. In galactose, Gal4 recruits components of the transcriptional machinery (1), including Srb10 and Kin28, which in turn phosphorylate Gal4 (2).

recruitment of RNA processing machinery. These findings reveal that a single transcription factor can interact with the Ub system in two different ways and suggest that activator destruction is required for pol II to transition from early post-initiation complexes to elongation complexes competent for mRNA production.

Results

Gal4 is an inducible transcription factor that regulates the expression of genes required for galactose utilization. The inducibility of Gal4 allowed us to probe how a native transcription factor is regulated by the Ub system under conditions in which it is inactive (raffinose) versus active (galactose). Even under activating conditions, however, not all Gal4 in the cell can be expected to participate in transcriptional activation. Reasoning that the stability of different pools of Gal4 may be regulated differently (Lipford and Deshaies, 2003), we developed a method to focus on the transcriptionally relevant pool of Gal4. Collectively, work from the Hopper (Mylin et al., 1990), Ptashne (Sadowski et al., 1991), and Sadowski (Hirst et al., 1999) laboratories has established that Gal4 is phosphorylated by pol II-associated kinases as a *consequence* of activating transcription. Although these phosphorylation events are not essential for activation by Gal4 (Sadowski et al., 1991), we reasoned that we could use them—and their effects on Gal4 mobility in SDS-PAGE—as a way to identify pools of Gal4 that had interacted with the pol II machinery.

We expressed HA epitope-tagged Gal4 from its own promoter and used Western blotting to visualize the Gal4 protein (Figure 1A). Consistent with previous reports, the pattern of Gal4 isoforms varies with carbon source. In the presence of raffinose, two Gal4 species are detected, which have been named isoforms "a" and "b" (Sadowski et al., 1991). In the presence of galactose, a third isoform, referred to as "c" (Gal4c), appears. Critically, Sadowski et al. (1991) have found that Gal4c depends on both the DNA binding and TAD functions of Gal4, suggesting that isoform c reflects a pool of Gal4 that is transcriptionally active. To extend these

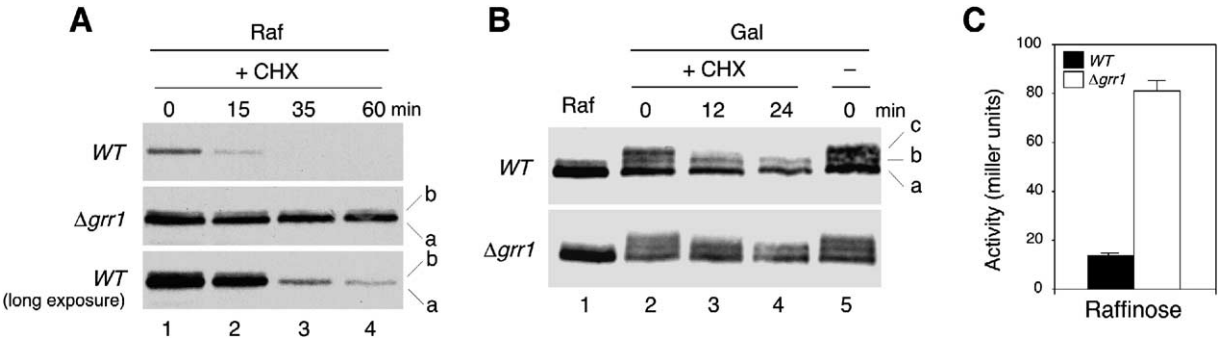

Figure 2. Grr1-Mediated Destruction of Gal4 Limits *GAL* Gene Activation

(A and B) Wild-type (JT1) or Δ*grr1* (JT11) yeast, expressing HA-tagged Gal4, were grown in raffinose-containing media and either not induced (A) or induced with galactose (B). "Cyclohexamide chase" was performed by addition of cyclohexamide (+CHX) or solvent (ethanol, –), taking aliquots of cultures at the indicated time points, and visualizing Gal4 protein by WB (Ab12CA5). In (A), a longer exposure of the WB from wild-type protein extracts is also presented. See also Figure S2.

(C) *GAL1-LacZ* reporter activity in rafinose media. β-galactosidase (β-gal) activity was assayed from JT1 and JT11 yeast growing in raffinose. Error bars represent the standard error of the mean (SEM).

findings, we asked how mutations in components of the transcriptional machinery affect Gal4c levels. This analysis (Figures 1A–1B) revealed that mutations which diminish Gal4 function result in decreased levels of Gal4c: deletion of genes encoding mediator subunits Gal11, Med2, and Pdg1, as well as genes encoding factors required for efficient transcription elongation—Hpr1, Paf1, and Rad6—produce a commensurate decrease in both Gal4c formation and Gal4 activity. Interestingly, deletion of genes encoding the TFIID-associated protein Bdf1 and the SAGA component Spt3 had little effect on Gal4 activity (Figure 1B), although they virtually eliminated isoform c formation. Taken with results of previous studies, these data suggest that phosphorylation events unique to isoform c occur as a consequence of activation of transcription by Gal4 but are not themselves required for Gal4 activity.

Several sites of phosphorylation within Gal4 have been reported, the most notable of which are serine 699 (S699), which is phosphorylated in vitro by Srb10 (Hirst et al., 1999), and serine 837 (S837), which is phosphorylated by Kin28 (Hirst et al., 1999). To characterize whether these phosphorylation events occur in our system, we raised polyclonal antibodies against phospho-S699 and phospho-S837 (Figure S1 available with this article online). Immunoprecipitation reactions demonstrated that the phospho-S699 antibodies recognize isoform c (Figure 1C, compare lanes 4 and 5), and that this recognition is inhibited by inclusion of the phosphorylated peptide used to generate the antibody (compare lanes 5 and 6). The phospho-S837 antibodies, in contrast, recognize isoform b in raffinose and isoforms b and c in galactose (not shown). Thus, although S699 phosphorylation is specific to isoform c, both Srb10- and Kin28-mediated phosphorylation events are present within the Gal4c species. Consistent with this finding, deletion of *Srb10* (Figure 1D), or chemical-genetic inhibition of Kin28 function (Liu et al., 2004); Figure 1E), eliminates isoform c formation. Together, these results—along with those of previous studies—strongly suggest that activation of transcription by Gal4 results in the Srb10- and Kin28-mediated phosphorylation of the protein at residues S699 and S837 (Figure 1F). We suggest that Gal4c corresponds to the pool of Gal4 that is either activating, or has activated, transcription. Isoforms a and b, in contrast, correspond to either inactive Gal4 or Gal4 that is activating in a functionally distinct manner from Gal4c.

Two Modes of Gal4 Proteolysis

We next examined the stability of the various Gal4 isoforms under inactive versus active conditions. We have measured Gal4 stability using both pulse-chase and protein synthesis shutoff techniques with similar results (*not shown*). Here, we use the synthesis shutoff approach, in which we measure decay of Gal4 following treatment of yeast with cyclohexamide. Analysis of Gal4 turnover reveals that, in the presence of raffinose (Figure 2A), Gal4 isoforms a and b are unstable and disappear with a half-life of ~20 min. In the presence of galactose, in contrast (Figure 2B, top panel), Gal4a and Gal4b are relatively stable (see also Figure S2), whereas Gal4c is unstable and disappears with a half-life of less than 5 min. Thus two distinct modes of Gal4 proteolysis can be described. Under non-inducing conditions, the Gal4a/b pool is destroyed fairly rapidly. Under inducing conditions, however, this pool of Gal4 is stable, but the active pool (Gal4c) is turned over rapidly.

The F Box Protein Grr1 Limits Gal4 Activity in Raffinose

To determine the functional consequence of Gal4a/b turnover in raffinose, we wished to block the destruction of these species. Although we have not demonstrated that Gal4 is destroyed by Ub-mediated proteolysis, the overwhelming majority of transcription factors studied to date are destroyed by this pathway. Indeed, we have consistently found that turnover of transcription factors in yeast depends on SCF-type Ub ligases (unpublished data), the substrate specificity factors for which are the "F box" proteins (Patton et al., 1998). We speculated, therefore, that destruction of Gal4a/b might

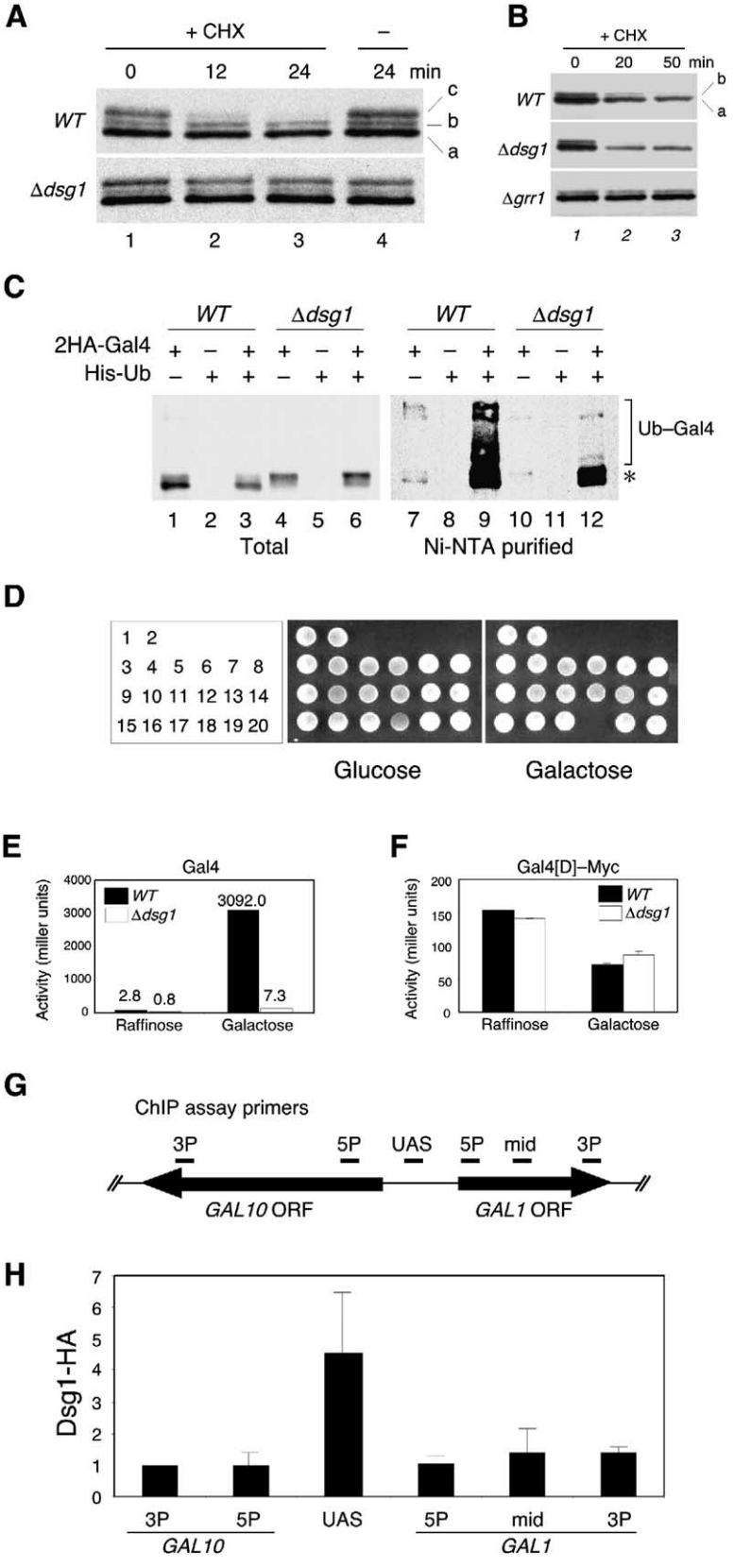

Figure 3. Dsg1 Is a Transcriptional Coactivator Required for Gal4c Turnover

(A and B) Dsg1 is required for Gal4c turnover. Cyclohexamide chase performed on WT (JN1), Δ*dsg1* (JN3), or Δ*grr1* (JN4) (all "+p2HG4") yeast grown in galactose- (A) or raffinose- (B) containing media.

(C) Dsg1 is required for Gal4 ubiquitylation in galactose. WT (JN1) and Δ*dsg1* (JN3) yeast were engineered to express HA-tagged Gal4 (p2HG4) either alone or in the presence of His-Ub. Total protein (lanes 1–6) was incubated with Ni-NTA resin, and ubiquitylated Gal4 species (Ub-Gal4) detected by WB (Ab12CA5). *Indicates background binding of Gal4 to the Ni-NTA resin; these species do not correspond to ubiquitylated Gal4 (not shown).

(D) *Dsg1*-null yeast cannot utilize galactose as a carbon source. Yeast bearing deletions of genes encoding 18 nonessential F box proteins were spotted onto plates containing glucose or galactose as the sole carbon source. The strains are 1: BY4742 (WT), 2: BY4739 (WT), 3: Δ*ylr097c*, 4: Δ*ufo1*, 5: Δ*ynl311c*, 6: Δ*dia2*, 7: Δ*rax1*, 8: Δ*ybr203w*, 9: Δ*ydr131c*, 10: Δ*ylr224w*, 11: Δ*yjl149w*, 12: Δ*ydr219c*, 13: Δ*rcy1*, 14: Δ*ydr306c*, 15: Δ*grr1*, 16: Δ*rev7*, 17: Δ*ybr280c*, 18: Δ*dsg1*, 19: Δ*ylr352w*, 20: Δ*ynl230c*.

(E and F) Dsg1 is specifically required for activation of a *GAL1-LacZ* reporter by the Gal4 activation domains. JN1 and JN3, expressing either full-length HA-tagged Gal4 (E) or a Gal4DBD-Myc TAD fusion protein (F; Gal4[D]–Myc), were grown in raffinose or galactose. β-galactosidase activity is expressed in Miller units. Error bars represent SEM.

(G) Amplicons used for ChIP analysis.

(H) Dsg1 associates with the *GAL1/10* UAS. Dsg1 was HA-epitope tagged at its carboxy terminus (WA1), cultures grown in galactose, and ChIP assay performed. Real-time PCR was used to calculate enrichment of the indicated amplicons in the Dsg1-tagged (WA1) versus untagged (W303-1a) strains. Error bars represent SEM.

require a specific F box protein. There are 21 genes encoding F box proteins in yeast; we screened the effects of deletions or temperature-sensitive mutations in 20 of these genes on Gal4a/b accumulation (not shown). Of the 20 mutants examined, only one—deleted for *Grr1*—displayed high levels of Gal4 protein in raffinose. As shown, deletion of *grr1* results in a dramatic stabilization of Gal4a/b (Figure 2A) but has little if any effect on destruction of Gal4c in galactose (Figure 2B). Consistent with the increased stability and steady-state levels of Gal4 in the *grr1*-null yeast, loss of Grr1 results in increased activation of Gal4 target genes. Indeed, in the absence of Grr1, we can detect significant activation of a *GAL1-LacZ* reporter under non-inducing conditions (Figure 2C). Similar effects have been reported for overexpression of Gal4 protein and probably result from titration of the Gal80 inhibitor (Ma and Ptashne, 1987). The ectopic activation of a Gal4 target gene in *grr1*-null cells suggests that Grr1-dependent turnover of Gal4 normally acts to restrict *GAL* gene activation under non-inducing conditions.

The F Box Protein Dsg1 Is Essential for Gal4 Activity

Loss of Grr1 did not affect the stability of Gal4c in galactose, revealing that a different pathway is responsible for the turnover of this species. To identify this pathway, we again asked whether a specific F box protein is required for Gal4c destruction. This analysis identified a single F box protein, we refer to here as Dsg1 ("*d*oes something to *G*al4"; YLR368W; also known as FLM1 and MDM30; Dimmer et al., 2002), that is required for Gal4c proteolysis. Deletion of *dsg1* stabilized Gal4c (Figure 3A)—but has no detectable effect on Gal4a/b turnover in raffinose (Figure 3B)—and blocks the accumulation of multi-ubiquitylated Gal4 species observed in galactose (Figure 3C). Thus Dsg1 is specifically required for the ubiquitylation and proteolysis of transcriptionally active Gal4 protein.

To determine the functional consequence of Dsg1-mediated Gal4c destruction, we asked whether deletion of *dsg1* alters Gal4 activity (Figures 3D–3E). In contrast to what we observed upon deletion of *grr1*, we found that deletion of *dsg1* virtually eliminated productive activation of transcription by Gal4. Yeast deleted for *Dsg1* are unable to use galactose as a carbon source (Figure 3D), a classic phenotype for deficiencies in *GAL* gene activation. Moreover, in the absence of Dsg1, activation of a *GAL1-LacZ* reporter gene is reduced to near background levels (Figure 3E). Interestingly, the requirement for Dsg1 in Gal4 activity seems to reside within the TAD of Gal4 because fusion of the Myc TAD to the Gal4 DNA binding domain (DBD) allowed activation of the *GAL1-LacZ* reporter independent of *Dsg1* status (Figure 3F). Finally, chromatin immunoprecipitation (ChIP) analysis (Figures 3G–3H) showed that Dsg1 associates with the *GAL1/10* locus and localizes to the UAS, where a cluster of Gal4 binding sites are located. Thus Dsg1 associates with the same region of promoter DNA as Gal4.

Taken together, we interpret the above data to indicate that Dsg1 is a chromatin bound transcriptional coactivator for Gal4 and functions—at least in part—to signal the ubiquitylation and destruction of active Gal4 protein.

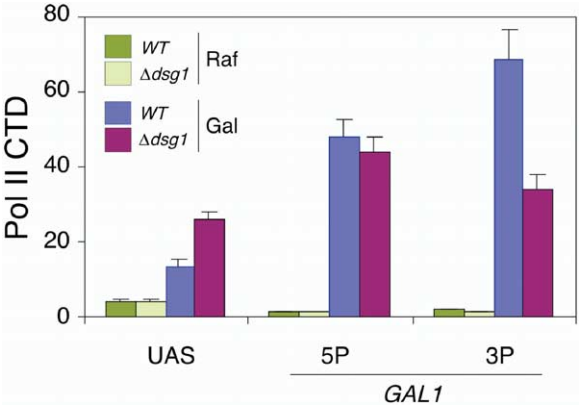

Figure 4. RNA pol II Is Efficiently Recruited to the *GAL1* Gene in the Absence of Dsg1

ChIP assay was used to measure association of the pol II-CTD (antibody 8WG16) with the *GAL1* gene in WT (JN1) or Δ*dsg1* (JN2) yeast grown in raffinose- or galactose-containing media. Error bars represent SEM.

Dsg1 Is Not Required for Efficient Transcription of Gal4 Target Genes

Deletion of genes encoding Hpr1 and Gal11 results in a decrease in both Gal4-dependent transcription and Gal4c formation (Figure 1B). Deletion of *Dsg1*, in contrast, decreased Gal4 activity but *increased* steady-state levels of Gal4c. If, as we suspect, isoform c is produced during interaction of Gal4 with pol II and its associated kinases, then we would predict that pol II is efficiently recruited to a *GAL* promoter in the absence of Dsg1. ChIP analysis confirmed this prediction (Figure 4); in *dsg1*-null yeast, galactose-dependent recruitment of the largest subunit of pol II to the *GAL1* promoter (UAS) was at least as high as observed in the wild-type control strain. Unexpectedly, however, we also found that pol II efficiently associated with sites throughout the *GAL1* open reading frame (ORF) in the absence of *dsg1*. The association of pol II with the 5′ and 3′ ends of the *GAL1* ORF was galactose inducible, and, although there was approximately 2-fold less pol II at the 3′ end of *GAL1* in *Δdsg1* yeast, the signal for pol II at this region was still ~40-fold higher than background levels. Thus, despite the profound effects of deletion of *dsg1* on galactose utilization and Gal4 activity, recruitment and distribution of pol II across a Gal4 target gene occurs efficiently.

We had assumed that the defect in *GAL1-LacZ* reporter activity observed in Δ*dsg1* cells (Figure 3E) results from defects in gene transcription. The finding that pol II is associated with the *GAL1* ORF, however, prompted us to ask whether *GAL* gene transcription still occurs in the Δ*dsg1* strain. Analysis of RNA species corresponding to the 3′ ends of the *GAL1-LacZ* reporter (Figure 5A) or the *GAL1* gene itself (Figure 5B) revealed that both genes are actively transcribed in the absence of Dsg1. Again, this effect is Gal4 TAD specific because

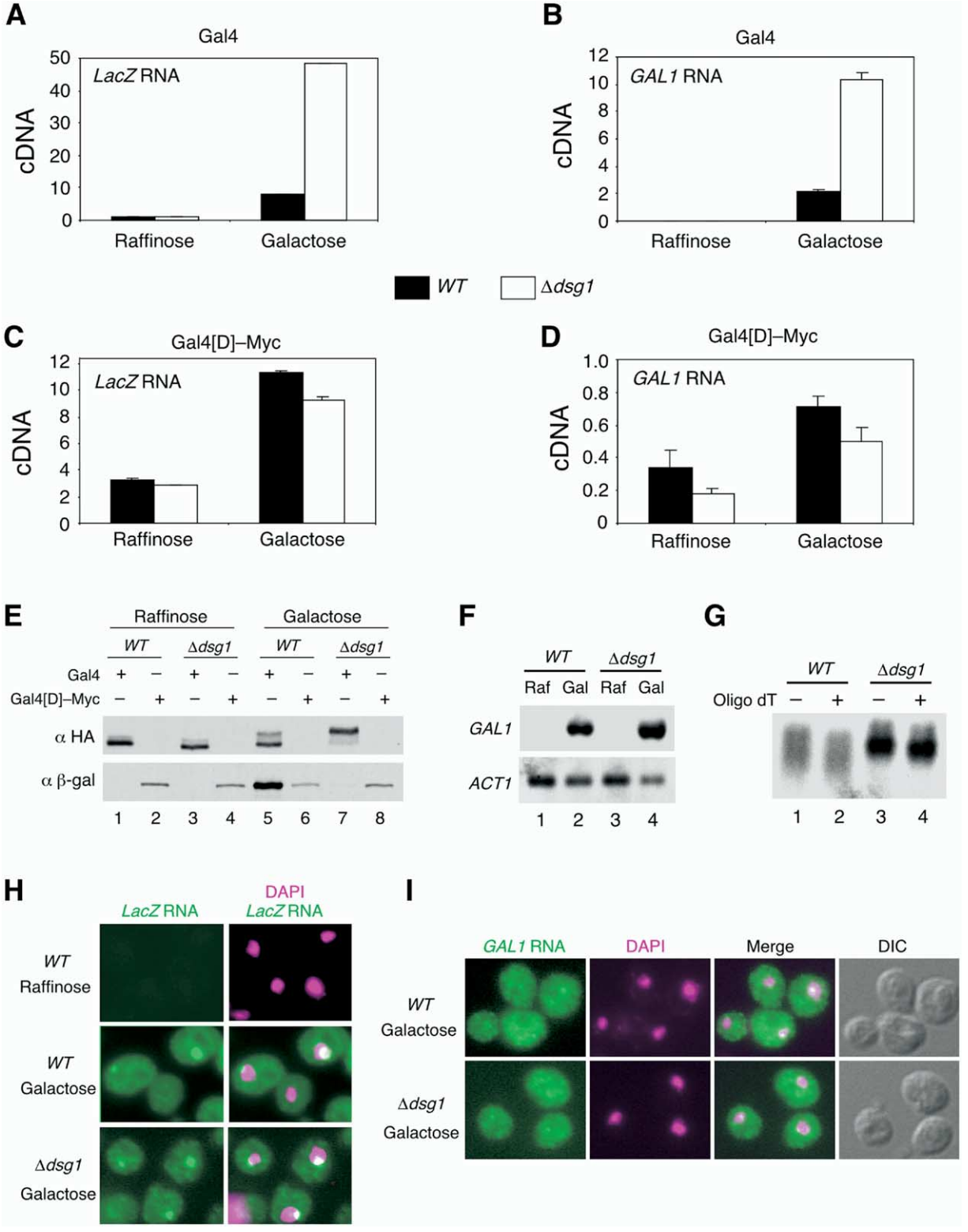

Figure 5. Gal4 Target Genes Are Efficiently Transcribed in the Absence of Dsg1

(A–D) Dsg1 is not required for induction of *GAL* gene RNAs. WT (JN1) or Δ*dsg1* (JN2) yeast, expressing either full-length HA-tagged Gal4 (A and B) or Gal4[D]-Myc (C and D), were grown in raffinose- or galactose-containing media. RNAs corresponding to *GAL1-LacZ* (A and C) or endogenous *GAL1* (B and D) were quantified by RT-PCR. Error bars represent SEM.

(E) Protein was simultaneously isolated from cultures assayed in (A)–(D), and WB used to detect expression of Gal4 (αHA) or β-galactosidase (β-gal).

activation by Gal4-Myc is insensitive to *dsg1* deletion (Figures 5C–5D). Our conclusion from these experiments is that disruption of Dsg1 uncouples RNA and protein levels from Gal4 target genes. This conclusion is best illustrated by comparing RNA and protein samples prepared in parallel from wild-type and Δ*dsg1* yeast carrying an integrated *GAL1-LacZ* reporter (Figures 5A and 5E): Compared to wild-type yeast, the Δ*dsg1* strain displays robust levels of *LacZ* RNA (Figure 5A), yet there is no detectable β-galactosidase protein expressed in these cells (Figure 5E, compare lanes 5 and 7). Compared to the pol II density across the *Gal1* gene (Figure 4), which was modestly reduced in the *Δdsg1* yeast, we consistently observed higher levels of Gal4 target RNAs in the absence of *dsg1*, indicating that overall rates of RNA synthesis or destruction are altered in *dsg1*-null cells.

Dsg1/Mdm30 has been shown to be required for maintenance of fusion-competent mitochondria (Fritz et al., 2003). At elevated temperatures (37°C), *dsg1/mdm30*-null yeast lose mitochondrial DNA, an event that would render yeast unable to utilize galactose as a carbon source. Although *dsg1*-null yeast do not completely lose mitochondrial DNA during the course of our experiments (data not shown), we tested whether loss of mitochondrial DNA could result in a disconnect between *GAL* gene RNA and protein levels. Deletion of the gene encoding Fzo1, a mitochondrial GTPase, results in defects in mitochondrial fusion and a loss of mitochondrial DNA (Rapaport et al., 1998). As expected, *fzo1*-null yeast could not grow on galactose-containing media (Figure S3A), but the defect in *GAL* gene induction occurred prior to activation of *GAL* gene transcription because, unlike *dsg1*-null cells, *fzo1*-null yeast display very low levels of *GAL1* RNA (Figure S3B). Thus loss of mitochondrial DNA is not responsible for the particular *GAL* gene phenotype we observe in *dsg1*-null yeast.

The disconnect between RNA and protein levels we observe in Δ*dsg1* yeast suggests that Dsg1 acts not to stimulate the efficiency of transcription per se but rather to control an essential aspect of the quality of resulting RNA species. This conclusion is supported by the finding that *GAL1* transcripts produced in *Δdsg1* cells are full-length, as judged by Northern blotting (Figure 5F), and polyadenylated, as judged by oligo(dT)-primed cDNA synthesis (Figure S4) and by oligo(dT)-directed RNase protection (Figure 5G), where differences in the size of RNase H cleavage products induced by hybridization of RNAs with oligo(dT) reveal polyadenylation status (Dower and Rosbash, 2002). Interestingly, compared with RNAs extracted from wild-type yeast, *GAL1* RNAs from *dsg1*-null yeast are less heterogeneous at their 3′ ends (compare lanes 1 and 3). The differences in 3′ ends suggests that, in part, Dsg1 may act to control utilization of poly(A) cleavage site selection at the *GAL1* gene.

Dsg1 Is Required for the Production of Functional Messenger RNAs

Because protein synthesis requires that transcripts move from the nucleus to the cytoplasm, we asked whether Dsg1 is required for export of *GAL* gene RNAs. We performed RNA fluorescent in situ hybridization (FISH) to visualize transcripts from *GAL1-LacZ* (Figure 5H) and *GAL1* (Figure 5I). As expected, in raffinose media no signal was detected (Figure 5H). In galactose media, we observed a strong signal in wild-type yeast in both the nucleus and the cytoplasm. Importantly, there was no detectable difference in the distribution of either *LacZ* or *GAL1* RNA in *dsg1*-null yeast, demonstrating that Dsg1 is not essential for nuclear export of *GAL* gene RNAs.

We next asked whether Dsg1 is required for association of *GAL1* RNA with translating ribosomes (Figure 6). We fractionated poly-ribosomes by sucrose gradient centrifugation (Du and Stillman, 2002; Figure 6A). Analysis of 25S and 18S ribosomal RNAs (Figure 6B) confirmed that fractions 4, 5, and 6 were enriched in rapidly sedimenting (>80S) ribosome particles. In wild-type cells, *GAL1* RNA efficiently cofractionated with this poly-ribosome fraction (Figure 6C, black bars), consistent with the concept that these RNAs are productively translated. In Δ*dsg1* cells, however, little if any *GAL1* RNA appeared in the polyribosome fraction (Figure 6C, white bars). Thus the failure of Gal4 to productively activate transcription in *dsg1*-null yeast can be traced to defects in the ability of *GAL* gene RNAs to engage translating ribosomes.

Dsg1 Is Required for Postrecruitment Phosphorylation of the pol II CTD

Production of a functional mRNA requires a series of processing events that occur cotranscriptionally (Maniatis and Reed, 2002) and are coordinated by phosphorylation of residues within the heptapeptide repeat (YSPTSPS) of the pol II CTD. Specifically, phosphorylation of serine residue 5 (Ser5) within the repeat—which is mediated by either Kin28 (Hengartner et al., 1998; Lu et al., 1992) or Bur1 (Murray et al., 2001)—occurs commensurate with transcription initiation (Kobor and Greenblatt, 2002) and is required for recruitment of the mRNA capping machinery (Cho et al., 2001; McCracken et al., 1997). Phosphorylation of serine residue 2, in contrast, is probably mediated by Ctk1 (Kobor and Greenblatt, 2002), is enriched toward the 3′ end of the ORF (Cho et al., 2001), and is thought to coordinate

(F) *GAL1* RNAs are full-length in the absence of Dsg1. Northern blotting.
(G) *GAL1* RNAs are polyadenylated in the absence of Dsg1 but have altered 3′ ends. RNA isolated from WT and *Δdsg1* cells was incubated with primer GAL1-mid-R (Table S3), either in the presence or absence of an oligo(dT) oligonucleotide, as indicated. DNA–RNA hybrids were cleaved with RNase H, and the resulting *GAL1* products detected by Northern blotting.
(H and I) Gal4 target gene RNAs are normally localized in the absence of Dsg1. RNA FISH was used to probe the localization of *LacZ* (H) or *GAL1* (I) RNAs. Nuclei were visualized by DAPI staining; yeast were visualized by differential interference contrast (DIC).

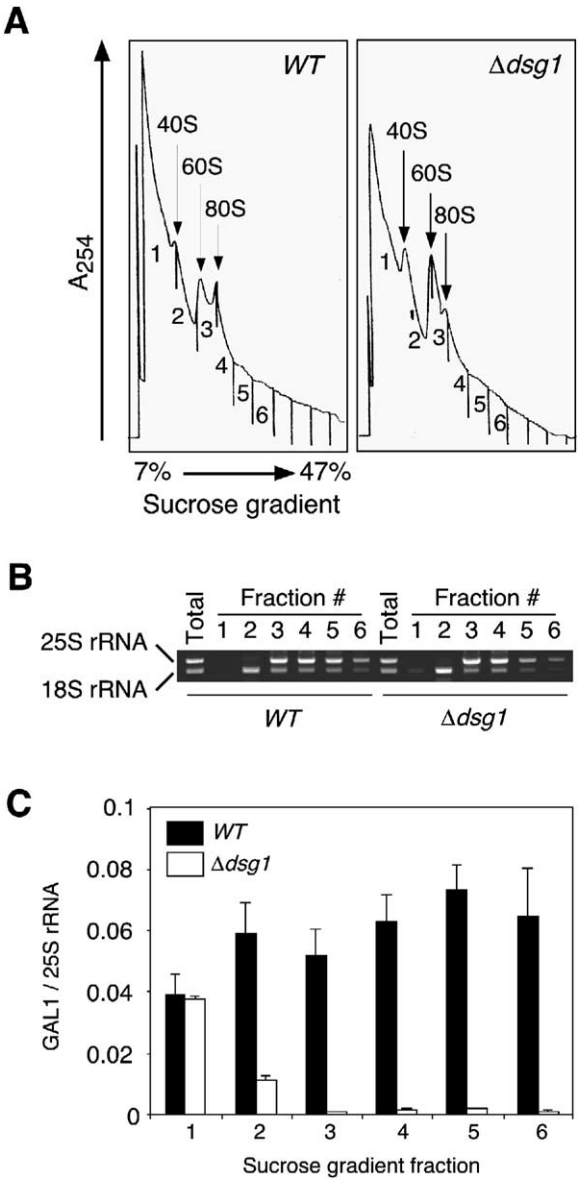

Figure 6. Dsg1 Is Required for Synthesis of Functional *GAL1* mRNAs

(A and B) Fractionation of translating ribosomes. Extracts from WT (JN1) or Δ*dsg1* (JN2) yeast were layered on 7%–47% sucrose gradient and ribosome species separated by ultracentrifugation. Absorbance of UV light at 254 nm (A), and the presence of both 18S and 25S ribosomal RNAs (B), were used to determine the profile of sedimenting ribosomes. Fractions 4–6 correspond to the polyribosome fraction.

(C) Analysis of polyribosome-associated *GAL1* RNA. RT-PCR analysis of *GAL1* RNAs in each fraction, normalized to levels of input 25S rRNA. Error bars represent SEM.

interaction of pol II with the termination and polyadenylation machinery (Ahn et al., 2004; Kim et al., 2004; Licatalosi et al., 2002). Because a failure to appropriately phosphorylate pol II could result in an uncapped, nonfunctional, RNA—and because the pol II CTD is required for proper mRNA 3′ end formation (which appears to be altered in *dsg1*-null cells; Figure 5G)—we therefore asked whether loss of Dsg1 alters the phosphorylation pattern of pol II at the *GAL1* gene (Figures 7A–7C).

We used ChIP, combined with phospho-specific antibodies against Ser5 and Ser2 of the CTD (Komarnitsky et al., 2000), to monitor distribution of phosphorylated pol II species across *GAL1*. In wild-type cells, robust Ser5 phosphorylation could be detected at the promoter, as well as the 5′ and 3′ ends of the *GAL1* gene (Figure 7B). In Δ*dsg1* cells, however, Ser5 phosphorylation can be detected at the promoter but is dramatically reduced within the *GAL1* ORF. Similar results were observed with the phospho-Ser2 antibody; deletion of *Dsg1* virtually eliminated detectable Ser2 phosphorylation across the *GAL1* (Figure 7C) and *GAL10* and *GAL7* genes (Figure S5). To probe whether this reduction in Ser5 or Ser2 phosphorylation was reflected in a global reduction in the level of these modifications, we performed Western blotting using the phospho-specific CTD antibodies (Figure 7D). This analysis showed that levels of both Ser2- and Ser5-phosphorylated CTD were reduced in the absence of *Dsg1* (compare lanes 2 and 4). For Ser5 phosphorylation, this defect is accounted for entirely by the lack of a productive *GAL* gene response because the effect of deleting *Dsg1* is matched by the effect of removing the Gal4 activator (compare lanes 1 and 4). For Ser2 phosphorylation, however, loss of Dsg1 is more detrimental than loss of Gal4 (compare lanes 1 and 3), and it is possible that the reduction in Ser2 phosphorylation observed in ChIP is the result of a general reduction in the levels of this modification. Although we cannot draw any conclusions about the specificity with which Ser2 phosphorylation is lost, we note that the Ser5 defect alone is sufficient to explain the nonfunctionality of *GAL1* transcripts in *Δdsg1* cells because cotranscriptional mRNA capping is required for translation (Gingras et al., 1999).

To ask whether other genes targets might be affected by deletion of *Dsg1*, we examined polymerase density across the *ADH1* gene (Figure 7E). This analysis showed that deletion of *Dsg1* resulted in a decrease in the total levels of pol II associated with the *ADH1* coding sequence (top panel); pol II density at the 5′ end of the coding sequence was reduced by 2-fold upon deletion of *Dsg1*, whereas density at the 3′ of the gene was reduced to background levels. A similar effect was observed at the *SUC2* and *HSP82* genes (data not shown). This effect on *ADH1* was dependent on Gal4—deletion of *Dsg1* had little effect on pol II density in the absence of the Gal4 activator (Figure 7E, lower panel)—suggesting that it may be the result of transcriptional squelching from accumulation of Gal4c protein (Figure 5E). Notably, this dominant, nonspecific effect is not responsible for the inability of yeast to grow on galactose media because addition of raffinose to galactose media allows *dsg1*-null yeast to grow at rates comparable to wild-control cells (Figure 7F).

To probe further into the Ser5 phosphorylation defect at the *GAL1* gene, we asked whether Bur1 is recruited to *GAL1* in the absence of Dsg1 (Figure 7E). We focused on Bur1 because it has been implicated in Ser5 phosphorylation (Keogh et al., 2003; Murray et al., 2001) and because the robust level of Gal4c formation in

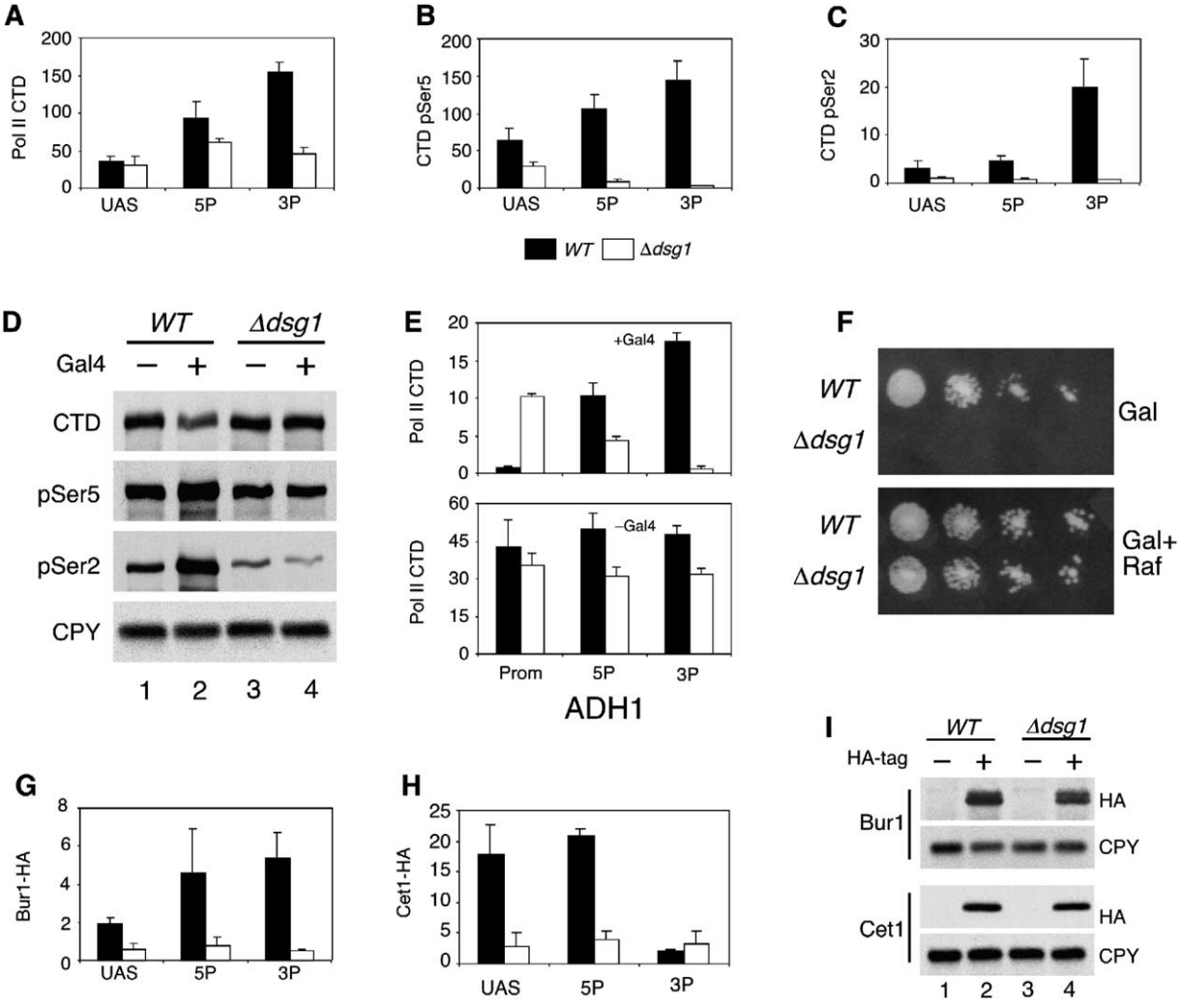

Figure 7. Dsg1 Is Required for Appropriate CTD Phosphorylation at the *GAL1* Gene

(A–C) Ser5 and Ser2 CTD phosphorylation are disrupted by loss of Dsg1. ChIP was used to determine the distribution of total CTD (A), and Ser5- (B) or Ser2- (C) phosphorylated CTD at the activated *GAL1* gene in WT (JN1) or Δ*dsg1* (JN3) yeast expressing HA-tagged Gal4 (p2HG4). Error bars represent SEM.

(D) Steady-state levels of pSer5 and pSer2. WB, showing the steady-state levels of pol II CTD (8WG16), pSer5 (H14), pSer2 (H5), and CPY loading control (A6428) in JN1 or JN2 yeast either containing or lacking plasmid p2HG4.

(E) Deletion of *Dsg1* alters pol II density on the *ADH1* gene. ChIP reactions analyzed in (A) were probed for the presence of *ADH1* promoter (prom) and 5′ (5P) and 3′ (3P) coding sequences. Top panel: ChIP reactions from yeast expressing Gal4 protein. Lower panel: ChIP reactions from yeast not expressing Gal4 protein.

(F) Growth of *dsg1*-null on galactose media is rescued by addition of raffinose. Ten-fold serial dilutions of WT or Δ*dsg1* yeast were spotted onto synthetic media plates containing either galactose, or galactose+raffinose, as the carbon source.

(G) Recruitment of the Bur1 kinase requires Dsg1. ChIP, showing recruitment of HA-tagged Bur1 to the activated *GAL1* gene in WT (JN6) or Δ*dsg1* (JN8) yeast expressing untagged Gal4 (pRJR197). Error bars represent SEM.

(H) Recruitment of Cet1 requires Dsg1. ChIP, showing recruitment of HA-tagged Cet1 to the activated *GAL1* gene in WT (JN7) or Δ*dsg1* (JN9) yeast expressing untagged Gal4 (pRJR197). Error bars represent SEM.

(I) Steady-state levels of HA-tagged Bur1 and Cet1. WB (12CA5 for HA-tagged proteins; A6428 for CPY).

Δ*dsg1* yeast (Figure 3A) suggests that Kin28 and Srb10 functionally interact with Gal4 in these cells (because both Kin28 and Srb10 are required for Gal4c formation [Figure 1]). In wild-type yeast, Bur1 associates mostly with the 5′ and 3′ ends of the *GAL1* ORF, indicating a predominant role in nonpromoter-associated Ser5 phosphorylation. In *dsg1*-null yeast, however, Bur1 fails to associate with the *GAL1* gene, an observation that is consistent with the finding that ORF-associated Ser5 phosphorylation is deficient in *dsg1*-null cells.

Finally, we asked whether an event that depends on Ser5 phosphorylation is also dependent on Dsg1. For this purpose, we measured recruitment of the capping enzyme component Cet1 (Figure 7F), which associates with pol II in response to Ser5 phosphorylation (Cho et al., 2001; McCracken et al., 1997). In wild-type cells,

we detected robust interaction of Cet1 with the *GAL1* promoter and 5′ ORF region. In *dsg1*-null cells, however, there was no detectable enrichment of Cet1 at promoter-proximal sites. Steady-state Cet1 levels, and those of Bur1, were not affected by *Dsg1* deletion (Figure 7G). The failure of Δ*dsg1* yeast to recruit Cet1 to *GAL1* demonstrates that Ser5 phosphorylation is functionally disturbed in these cells and supports the concept that Dsg1 plays a role in regulating cotranscriptional processing of Gal4 target RNAs.

Discussion

A characteristic feature of many transcriptional activators is their metabolic instability, which is generally thought to limit their function. Yet it has become clear that the Ub system can act to positively regulate activators and other components of the transcriptional machinery (Lipford and Deshaies, 2003; Muratani and Tansey, 2003). To reconcile these observations, and to learn more about how Ub controls the activity of a native transcription factor, we have studied Gal4. We find that Gal4 stability is regulated by two distinct mechanisms with distinct consequences for transcription. Destruction of Gal4 via Grr1 acts to limit Gal4 accumulation and prevent ectopic *GAL* gene activation. Destruction of Gal4 via Dsg1, in contrast, is important for productive *GAL* gene activation. Surprisingly, the step in Gal4-mediated activation that is dependent on Dsg1 is not transcription per se, but appropriate RNA pol II phosphorylation events that coordinate transcription with pre-messenger RNA processing.

Phosphorylation of Gal4 by RNA pol II-Associated Kinases

Previous work from a number of laboratories has demonstrated that Gal4 is phosphorylated as a consequence of activating transcription. Our findings confirm these observations and suggest that phosphorylation of Gal4, at least at residue S699, is used as a cellular mechanism to "mark" pools of Gal4 that have stimulated transcription. A similar situation has previously been reported for GCN4 (Chi et al., 2001), suggesting that phosphorylation by basal factor kinases may be a general bookkeeping mechanism to signal the history of transcriptional activators. For GCN4, Srb10-mediated phosphorylation clearly leads to recognition by the F box protein Cdc4 and Ub-mediated GCN4 destruction (Chi et al., 2001), and it is possible that a similar scenario applies for Gal4c and Dsg1. We should note that although we cannot formally exclude the possibility that Gal4c is *dephosphorylated* in a Dsg1-dependent manner, we think this unlikely because of the observation that declining levels of Gal4c are not accompanied by an increase in the levels of Gal4a/b (Figures 2B and 3B) and because Dsg1 is an F box protein that is required for Gal4 ubiquitylation (Figure 3C).

Two Modes of Gal4 Proteolysis

Our data establish that at least two pathways control Gal4 stability. Under non-inducing conditions Gal4 is destroyed fairly rapidly, with a half-life of ~20 min. This destruction depends on the F box protein Grr1. We suggest that this mode of Gal4 destruction limits inappropriate *GAL* gene activation because disruption of *Grr1* results in the accumulation of Gal4 and ectopic activation of a *GAL1-LacZ* reporter gene. Interestingly, Grr1 was identified as a gene required for glucose-mediated gene repression (Bailey and Woodword, 1984) and was shown to act, in part, via positive regulation of the glucose-specific Mig1 repressor (Lutfiyya et al., 1998). Our data, implicating a role for Grr1 in the regulation of Gal4 stability, reveal a second mechanism through which Grr1 can limit *GAL* activation and illuminate how a single Ub ligase can regulate gene expression through action on multiple target proteins.

Under inducing conditions, the Grr1-dependent pathway of Gal4 proteolysis seems to be inactivated because Gal4 isoforms a and b are stable when yeast are grown in galactose media. It is tempting to speculate that stabilization of Gal4a/b in galactose facilitates transcription by increasing the pool of Gal4 that is available to activate gene expression. Indeed, given the rapid Dsg1-mediated turnover of Gal4c, and the possibility that active Gal4 is destroyed with each cycle of productive transcription, it is likely that this stabilization is important for maintaining appropriate levels of Gal4 at target gene promoters.

Once Gal4 is engaged in activation, its ubiquitylation and destruction are under the control of Dsg1. This conclusion is supported by the effects of *Dsg1* deletion on Gal4 ubiquitylation and Gal4c stability and by the finding that Dsg1 associates with the UAS regulatory region of the *GAL1/10* locus. The actions of Dsg1, in contrast to Grr1, are clearly stimulatory because Gal4 is unable to productively activate transcription in Δ*dsg1* cells. Indeed, as discussed below, Dsg1 functions as an essential "coactivator" for the Gal4 protein.

Previously, a unified model for how transcriptional activators are regulated by ubiquitylation has been difficult to propose. In some cases, such as p53 (Haupt et al., 1997), it is clear that ubiquitylation leads to proteolysis and inhibition of activator function. In other cases, however, ubiquitylation leads to proteolysis and *enhanced* activator function (Salghetti et al., 2001). Our finding that Gal4 stability is regulated by at least two distinct F box proteins, with two distinct consequences for transcription, provides a way to reconcile these observations. We propose that other transcription factors may have more than one mode of regulation by the Ub proteasome system: one that is independent of transcription and limits factor activity, and another that is coupled to transcription and performs an essential process in gene activation. In this scenario, although the transcription-independent pathway of proteolysis could be regulated by environmental factors, the transcription-coupled mechanism would be inevitably linked to activity. With Gal4 this predicts that Dsg1-dependent ubiquitylation and proteolysis are not sugar linked but occur whenever Gal4 is active, such as in raffinose conditions in *grr1*-null yeast.

Dsg1 Is a Transcriptional Coactivator Required Appropriate pol II Phosphorylation

Numerous Ub ligases have been described as having transcriptional coactivator function. Our observation

that Dsg1 is essential for productive activation of transcription by Gal4 gave us the opportunity to study how a Ub ligase coactivator can work. We find that, in the absence of Dsg1, Gal4 can efficiently recruit pol II to target genes, that these genes are transcribed, but that resulting RNAs are not translated. Although we have not demonstrated that *GAL* RNAs are inappropriately processed in the absence of *dsg1*, the functional defects in these RNAs can be attributed to a failure of RNA pol II to receive appropriate phosphorylation events that in turn are required for cotranscriptional RNA processing. Thus Dsg1 does not control basic processes required for RNA transcription—which may be one reason why a requirement for Ub ligases in transcription in vitro has not been demonstrated—but instead controls events governing mRNA quality and activity.

Our model for how Dsg1 connects Gal4c destruction and RNA pol II phosphorylation is as follows. Based on our demonstration that promoter-associated Ser5 phosphorylation occurs with reasonable efficiency in Δ*dsg1* cells (Figure 7), we suggest that transcription factor recruitment and initiation of transcription occur independently of Gal4 ubiquitylation and Dsg1 activity. As RNA pol II continues to transcribe, however, the initiation-competent pol II complexes must be remodeled to strip away factors required for preinitiation complex assembly and to allow the kinases (e.g., Bur1) and RNA processing machinery (e.g., Cet1) required for productive mRNA synthesis to associate with the transcribing polymerase. This transition phase is the period at which pol II is briefly stalled to allow nucleation of productive elongation complexes (Mandal et al., 2004; Pei et al., 2003). We propose that Dsg1-mediated destruction of Gal4 is an essential part of initiation complex disassembly. We suggest that, in the absence of Dsg1, Gal4c is stable, the transition does not occur, and immature RNA pol II complexes transcribe nonprocessed, nonfunctional RNAs. In support of this model, we note that mutations in the ISW1 ATPase, which is implicated in the transition from initiation to elongation, produce a very similar phenotype to that seen in *dsg1*-null yeast. (Morillon et al., 2003). Note that, at this point, we cannot exclude the possibility that Dsg1 has targets in addition to Gal4 and that destruction of these targets is also required for the functionality of Gal4-dependent mRNAs.

The observation that Gal4c stability is connected to cotranscriptional RNA processing reveals that activators, and their regulatory molecules, control an unexpected step in RNA synthesis, where RNA transcripts are signaled to become functional *messenger* RNAs. This activity provides a level of transcriptional control that could be quite effective. By influencing the ubiquitylation status of an activator—either by disabling the relevant Ub ligase or perhaps blocking sites of ubiquitylation (e.g., by SUMOylating critical lysine residues)—the cell could rapidly convert a transcriptional activator into a de facto repressor, generating high levels of nonfunctional RNAs while at the same time keeping the promoter open and active to allow a rapid return to functional mRNA synthesis when appropriate. Curiously, if such a level of regulation does exist, analysis of steady-state RNA levels may not always be a reliable measure of gene activity.

Finally, we suggest that Ub-mediated proteolysis may play important roles in other transitions required for the "transcription cycle" (reviewed in Kobor and Greenblatt, 2002). As pol II transcribes genes, its complement of interacting proteins must change not only during the elongation checkpoint but also during events involved in termination and 3′ end processing, and when pol II encounters a site of DNA damage. The involvement of proteolysis in these transitions would not only actively drive disassembly of these complexes but would ensure that events important for RNA processing and polymerase disengagement occur in an irreversible manner. Perhaps, therefore, as in the cell cycle, proteolysis provides an unequivocal signal of directionality to the transcription process.

Experimental Procedures

Yeast and Plasmids

The *S. cerevisiae* strains, plasmids, and culture conditions used in this study are listed as Supplemental Data. Gal4 was expressed under the control of its own promoter from the *ARS/CEN* vector pRJR197 (Wu et al., 1996), modified to include a double HA-epitope tag at the amino terminus of Gal4 (p2HG4). For *GAL* gene induction, yeast were grown in synthetic medium with 2% raffinose to an OD_{600} of ~0.5 and induced either by addition of 2% galactose (Figures 1 and 2) or by transferring cells into fresh media containing 2% galactose (other experiments). Yeast were induced for 3 hr prior to analysis. Chemical-genetic inhibition of Kin28 was performed by treating strain Kin28 as (Liu et al., 2004) with 5 μM 1-napthyl-PP1 (1-NA; Liu et al., 2004) for 60 min prior to protein harvest.

Antibodies

Phospho-specific Gal4 antibodies were raised, purified, and tested as described in the Supplemental Data. Anti-Gal4 antibody was provided by W. Herr (CSHL); anti-HA antibody 12CA5 was provided by the CSHL antibody facility; anti-β-galactosidase antibody was from Roche (1083082); anti-CTD antibodies were from Covance (CTD: 8WG16; pSer2: H5; pSer5: H14); anti-CPY antibody was from Molecular Probes (A6428).

Protein Assays

Details of protein extraction, Western blotting, immunoprecipitation, ubiquitylation, and β-galactosidase reporter assays are presented as Supplemental Data. Gal4 stability was measured by adding cyclohexamide (50 μg/ml) to yeast growing under appropriate conditions, taking samples at the indicated time points, and analyzing steady-state HA-Gal4 levels by Western blotting with 12CA5. For Gal4 ubiquitylation, yeast JN1 (*DSG1*) and JN3 (Δ*dsg1::LEU2*), each carrying p2HG4, were transformed with the vector pUB221 (Yaglom et al., 1995), which expresses His6-tagged Ub under the control of the *CUP1* promoter. Yeast were induced with galactose and copper sulfate for 3 hr, polyhistidine-tagged proteins recovered under strongly denaturing conditions, and ubiquitylated Gal4 proteins detected by Western blotting with 12CA5.

Chromatin Immunoprecipitation

ChIP assays were performed essentially as described (Komarnitsky et al., 2000). Following immunoprecipitation, DNA was recovered by ethanol precipitation, and coprecipitating DNAs detected by real-time, quantitative PCR. Primer sets are described in Table S3. To calculate specific binding of target proteins to *GAL* genes, the signal from each *GAL* gene primer set was normalized to the signal from the 25S ribosomal DNA locus, and this value further normalized to the same ratio from input DNA. When HA-tagged proteins were used for IP, we also normalized these values to those from parallel ChIP reactions performed in untagged control yeast strains.

RNA Analyses

Details of RNA quantification, RNA FISH, Northern blotting, and RNaseH protection are presented as Supplemental Data. For analysis of RNA levels, RNA was extracted using the RNeasy Mini Kit (*Qiagen*), reverse-transcribed, and quantified by RT-PCR. Steady-state levels of *GAL1* and *LacZ* RNAs were normalized to those of *ACT1*. For RNA FISH, we used a protocol published online at http://www.singerlab.org/protocols. The RNase H protection assay was performed as described (Dower and Rosbash, 2002).

Ribosome Preparation

The translating ribosome fractionation was performed essentially as described (Du and Stillman, 2002), and as detailed in Supplemental Data. Briefly, cells were induced with galactose for 60 min, cyclohexamide added to inhibit protein synthesis, and cell extracts immediately prepared by glass bead beating. Lysates were layered on a continuous 7%–47% sucrose gradient and separated by ultracentrifugation. Distribution of RNAs across the gradient was monitored by measuring absorbance at 254 nm. Ribosomes were dissociated by addition of EDTA, and RNAs recovered by phenol-chloroform extraction, lithium precipitation, and a subsequent round of ethanol precipitation. Ribosome integrity was determined by detection of both 18S and 25S RNAs by agarose gel electrophoresis. *GAL1* RNAs present in the polyribosome fraction were quantified by RT-PCR.

Supplemental Data

Supplemental Data include Experimental Procedures, five figures, and three tables and can be found with this article online at http://www.cell.com/cgi/content/full/120/6/887/DC1/.

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References

Aberle, H., Bauer, A., Stappert, J., Kispert, A., and Kemler, R. (1997). Beta-catenin is a target for the ubiquitin-proteasome pathway. EMBO J. *16*, 3797–3804.

Ahn, S.H., Kim, M., and Buratowski, S. (2004). Phosphorylation of serine 2 within the RNA polymerase II C-terminal domain couples transcription and 3′ end processing. Mol. Cell *13*, 67–76.

Bailey, R.B., and Woodword, A. (1984). Isolation and characterization of a pleiotropic glucose repression resistant mutant of Saccharomyces cerevisiae. Mol. Gen. Genet. *193*, 507–512.

Bastien, J., and Rochette-Egly, C. (2004). Nuclear retinoid receptors and the transcription of retinoid-target genes. Gene *328*, 1–16.

Chi, Y., Huddleston, M.J., Zhang, X., Young, R.A., Annan, R.S., Carr, S.A., and Deshaies, R.J. (2001). Negative regulation of Gcn4 and Msn2 transcription factors by Srb10 cyclin-dependent kinase. Genes Dev. *15*, 1078–1092.

Cho, E.J., Kobor, M.S., Kim, M., Greenblatt, J., and Buratowski, S. (2001). Opposing effects of Ctk1 kinase and Fcp1 phosphatase at Ser 2 of the RNA polymerase II C-terminal domain. Genes Dev. *15*, 3319–3329.

Dimmer, K.S., Fritz, S., Fuchs, F., Messerschmitt, M., Weinbach, N., Neupert, W., and Westermann, B. (2002). Genetic basis of mitochondrial function and morphology in Saccharomyces cerevisiae. Mol. Biol. Cell *13*, 847–853.

Dower, K., and Rosbash, M. (2002). T7 RNA polymerase-directed transcripts are processed in yeast and link 3′ end formation to mRNA nuclear export. RNA *8*, 686–697.

Du, Y.C., and Stillman, B. (2002). Yph1p, an ORC-interacting protein: potential links between cell proliferation control, DNA replication, and ribosome biogenesis. Cell *109*, 835–848.

Fritz, S., Weinbach, N., and Westermann, B. (2003). Mdm30 is an F-box protein required for maintenance of fusion-competent mitochondria in yeast. Mol. Biol. Cell *14*, 2303–2313.

Gingras, A.C., Raught, B., and Sonenberg, N. (1999). eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. Annu. Rev. Biochem. *68*, 913–963.

Haupt, Y., Maya, R., Kazaz, A., and Oren, M. (1997). Mdm2 promotes the rapid degradation of p53. Nature *387*, 296–299.

Hengartner, C.J., Myer, V.E., Liao, S.M., Wilson, C.J., Koh, S.S., and Young, R.A. (1998). Temporal regulation of RNA polymerase II by Srb10 and Kin28 cyclin-dependent kinases. Mol. Cell *2*, 43–53.

Hirst, M., Kobor, M.S., Kuriakose, N., Greenblatt, J., and Sadowski, I. (1999). GAL4 is regulated by the RNA polymerase II holoenzyme-associated cyclin-dependent protein kinase SRB10/CDK8. Mol. Cell *3*, 673–678.

Kadonaga, J.T. (2004). Regulation of RNA polymerase II transcription by sequence-specific DNA binding factors. Cell *116*, 247–257.

Keogh, M.C., Podolny, V., and Buratowski, S. (2003). Bur1 kinase is required for efficient transcription elongation by RNA polymerase II. Mol. Cell. Biol. *23*, 7005–7018.

Kim, M., Ahn, S.H., Krogan, N.J., Greenblatt, J.F., and Buratowski, S. (2004). Transitions in RNA polymerase II elongation complexes at the 3′ ends of genes. EMBO J. *23*, 354–364.

Kobor, M.S., and Greenblatt, J. (2002). Regulation of transcription elongation by phosphorylation. Biochim. Biophys. Acta *1577*, 261–275.

Komarnitsky, P., Cho, E.J., and Buratowski, S. (2000). Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes Dev. *14*, 2452–2460.

Licatalosi, D.D., Geiger, G., Minet, M., Schroeder, S., Cilli, K., McNeil, J.B., and Bentley, D.L. (2002). Functional interaction of yeast pre-mRNA 3′ end processing factors with RNA polymerase II. Mol. Cell *9*, 1101–1111.

Lipford, J.R., and Deshaies, R.J. (2003). Diverse roles for ubiquitin-dependent proteolysis in transcriptional activation. Nat. Cell Biol. *5*, 845–850.

Liu, Y., Kung, C., Fishburn, J., Ansari, A.Z., Shokat, K.M., and Hahn, S. (2004). Two cyclin-dependent kinases promote RNA polymerase II transcription and formation of the scaffold complex. Mol. Cell. Biol. *24*, 1721–1735.

Lu, H., Zawel, L., Fisher, L., Egly, J.M., and Reinberg, D. (1992). Human general transcription factor IIH phosphorylates the C-terminal domain of RNA polymerase II. Nature *358*, 641–645.

Lutfiyya, L.L., Iyer, V.R., DeRisi, J., DeVit, M.J., Brown, P.O., and Johnston, M. (1998). Characterization of three related glucose repressors and genes they regulate in Saccharomyces cerevisiae. Genetics *150*, 1377–1391.

Ma, J., and Ptashne, M. (1987). The carboxy-terminal 30 amino acids of GAL4 are recognized by GAL80. Cell *50*, 137–142.

Mandal, S.S., Chu, C., Wada, T., Handa, H., Shatkin, A.J., and Reinberg, D. (2004). Functional interactions of RNA-capping enzyme with factors that positively and negatively regulate promoter escape by RNA polymerase II. Proc. Natl. Acad. Sci. USA *101*, 7572–7577.

Maniatis, T., and Reed, R. (2002). An extensive network of coupling among gene expression machines. Nature *416*, 499–506.

McCracken, S., Fong, N., Rosonina, E., Yankulov, K., Brothers, G., Siderovski, D., Hessel, A., Foster, S., Shuman, S., and Bentley, D.L. (1997). 5′-Capping enzymes are targeted to pre-mRNA by binding

to the phosphorylated carboxy-terminal domain of RNA polymerase II. Genes Dev. *11*, 3306–3318.

Molinari, E., Gilman, M., and Natesan, S. (1999). Proteasome-mediated degradation of transcriptional activators correlates with activation domain potency in vivo. EMBO J. *18*, 6439–6447.

Morillon, A., Karabetsou, N., O'Sullivan, J., Kent, N., Proudfoot, N., and Mellor, J. (2003). Isw1 chromatin remodeling ATPase coordinates transcription elongation and termination by RNA polymerase II. Cell *115*, 425–435.

Muratani, M., and Tansey, W.P. (2003). How the ubiquitin-proteasome system controls transcription. Nat. Rev. Mol. Cell Biol. *4*, 192–201.

Murray, S., Udupa, R., Yao, S., Hartzog, G., and Prelich, G. (2001). Phosphorylation of the RNA polymerase II carboxy-terminal domain by the Bur1 cyclin-dependent kinase. Mol. Cell. Biol. *21*, 4089–4096.

Mylin, L.M., Johnston, M., and Hopper, J.E. (1990). Phosphorylated forms of GAL4 are correlated with ability to activate transcription. Mol. Cell. Biol. *10*, 4623–4629.

Ostendorff, H.P., Peirano, R.I., Peters, M.A., Schluter, A., Bossenz, M., Scheffner, M., and Bach, I. (2002). Ubiquitination-dependent cofactor exchange on LIM homeodomain transcription factors. Nature *416*, 99–103.

Patton, E.E., Willems, A.R., and Tyers, M. (1998). Combinatorial control in ubiquitin-dependent proteolysis: don't Skp the F-box hypothesis. Trends Genet. *14*, 236–243.

Pei, Y., Schwer, B., and Shuman, S. (2003). Interactions between fission yeast Cdk9, its cyclin partner Pch1, and mRNA capping enzyme Pct1 suggest an elongation checkpoint for mRNA quality control. J. Biol. Chem. *278*, 7180–7188.

Rapaport, D., Brunner, M., Neupert, W., and Westermann, B. (1998). Fzo1p is a mitochondrial outer membrane protein essential for the biogenesis of functional mitochondria in Saccharomyces cerevisiae. J. Biol. Chem. *273*, 20150–20155.

Russell, S.J., Reed, S.H., Huang, W., Friedberg, E.C., and Johnston, S.A. (1999). The 19S regulatory complex of the proteasome functions independently of proteolysis in nucleotide excision repair. Mol. Cell *3*, 687–695.

Sadowski, I., Niedbala, D., Wood, K., and Ptashne, M. (1991). GAL4 is phosphorylated as a consequence of transcriptional activation. Proc. Natl. Acad. Sci. USA *88*, 10510–10514.

Salghetti, S.E., Caudy, A.A., Chenoweth, J.G., and Tansey, W.P. (2001). Regulation of transcriptional activation domain function by ubiquitin. Science *293*, 1651–1653.

Salghetti, S.E., Muratani, M., Wijnen, H., Futcher, B., and Tansey, W.P. (2000). Functional overlap of sequences that activate transcription and signal ubiquitin-mediated proteolysis. Proc. Natl. Acad. Sci. USA *97*, 3118–3123.

Sun, Z.W., and Allis, C.D. (2002). Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. Nature *418*, 104–108.

Terrell, J., Shih, S., Dunn, R., and Hicke, L. (1998). A function for monoubiquitination in the internalization of a G protein-coupled receptor. Mol. Cell *1*, 193–202.

Wu, Y., Reece, R.J., and Ptashne, M. (1996). Quantitation of putative activator-target affinities predicts transcriptional activating potentials. EMBO J. *15*, 3951–3963.

Yaglom, J., Linskens, M.H., Sadis, S., Rubin, D.M., Futcher, B., and Finley, D. (1995). p34Cdc28-mediated control of Cln3 cyclin degradation. Mol. Cell. Biol. *15*, 731–741.