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review

Yeast Gal4: a transcriptional paradigm revisited

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During the past two decades, the yeast Gal4 protein has been used as a model for studying transcriptional activation in eukaryotes. Many of the properties of transcriptional regulation first demonstrated for Gal4 have since been shown to be reiterated in the function of several other eukaryotic transcriptional regulators. Technological advances based on the transcriptional properties of this factor—such as the two-hybrid technology and Gal4-inducible systems for controlled gene expression—have had far-reaching influences in fields beyond transcription. In this review, we provide an updated account of Gal4 function, including data from new technologies that have been recently applied to the study of the GAL network.

Keywords: Gal4; RNA polymerase II; transcription; yeast

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Introduction

Transcriptional regulation of protein-coding genes in eukaryotes requires the combined action of many proteins impinging on the promoter region of a gene. In addition to RNA polymerase II, these proteins fall into the broad classes of activator or repressor proteins—coactivator or corepressor complexes—and general transcription factors. Several of these proteins are conserved between yeast and mammals, thus studies in the yeast *Saccharomyces cerevisiae* have been particularly useful for the study of gene transcription by RNA polymerase II. One of the earliest model systems for studying transcriptional regulation was that of the galactose-mediated induction of gene expression in yeast, which is under the control of the transcriptional activator Gal4. Pioneering studies on Gal4 from the Ptashne laboratory shaped our conceptual understanding of transcriptional activation in eukaryotes (reviewed in Ptashne & Gann, 2002). These studies showed the modular nature of the DNA-binding and activation domains of transcriptional activators and, as Gal4 could activate transcription when expressed in mammalian cells, that the activation mechanism is conserved in eukaryotes. Ultimately, they showed that the function of the activator is to recruit the transcriptional machinery to the promoter, as fusions of the Gal4 DNA-binding domain to targets in the transcriptional machinery could function as activators in their own right.

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The GAL regulon

The GAL genes are required for the growth of yeast on galactose and they comprise structural (*GAL1*, *GAL10*, *GAL2* and *GAL7*) and regulatory (*GAL4*, *GAL80* and *GAL3*) genes. The products of the GAL genes are required for the transport of galactose into the cell and its metabolism through the glycolytic pathway. Induction of the GAL structural genes by galactose is dependent on the transcriptional activator Gal4 that operates through an upstream activating sequence (UAS_{GAL}) present in their promoters. The number of UAS_{GAL} sites and their relative affinity for Gal4 vary among the *GAL* genes and this leads to differential activation (Lohr *et al*, 1995). The UAS_{GAL} is sufficient for mediating galactose induction through Gal4 when fused to a heterologous gene, and this property has been widely applied to the generation of inducible heterologous gene expression systems.

A genome-wide analysis of promoters bound by Gal4 and induced by galactose found that in addition to the previously identified GAL genes, *MTH1*, *PCL10* and *FUR4* also belong to the GAL regulon (Ren *et al*, 2000). These genes are not required for galactose metabolism, but rather for global adaptation to growth on galactose. For example, the induction of *MTH1*, a repressor of glucose transporter gene expression, results in the inhibition of glucose transport when glucose is depleted.

Approximately 300 potential Gal4-binding sites have been identified throughout the yeast genome. Apart from promoter regions, sites can be found in open reading frames (ORFs), and such sites in the acetyl-CoA carboxylase 1 gene (*ACC1*) can bind Gal4 and lead to decreased *ACC1* expression in the presence of galactose (Li & Johnston, 2001). However, Gal4 sites in the *ACC1* ORF are not conserved in yeast species and their elimination has no consequence for growth on galactose. It has been proposed that they are 'noise' in the occurrence of Gal4-binding sites in the genome (Li & Johnston, 2001). What determinants influence the binding or non-binding of Gal4 to the various binding sites in the genome is not yet clear. Chromatin structure might regulate the accessibility of Gal4 to some potential sites. In the case of *GAL1–GAL10*, *GAL7* and *GAL80* promoters, the UAS element is found in a nucleosome-free position regardless of the carbon source (Lohr, 1997). By contrast, nucleosomes are positioned over the TATA boxes and initiation sites and these are disrupted after galactose induction in a Gal4-dependent manner (Lohr, 1997). Other mechanisms that could influence the occupancy of the UAS_{GAL} by Gal4 include the binding affinities of the individual sites, their number and the vicinity of other regulatory regions.

The Gal4–Gal80 regulatory axis

Gal4 is an 881-amino-acid protein with a Zn–Cys binuclear cluster-type DNA-binding domain, a linker domain, a dimerization domain and two acidic activation domains (ARI and ARII; Lohr *et al*, 1995). The first *gal*$^-$ mutants to be isolated contained mutations in residues throughout the molecule, but with significant clustering in the DNA-binding domain (Johnston & Dover, 1987). The consensus Gal4-binding site is a 17mer of sequence 5′-CGG-N11-CCG-3′. The crystal structure of the minimal DNA-binding domain (amino acids 1–65) in complex with a consensus UAS shows that Gal4 binds as a dimer (Marmorstein *et al*, 1992). The Gal4 Zn–Cys domains contact the CGG elements directly, whereas the linker and dimerization domains interact with the phosphate backbone of the spacer residues in the 17mer. The CGG residues and the exact length of the spacer region are crucial to Gal4 binding (Liang *et al*, 1996). Domain-swap experiments with activators of the same family (Gal4, Put3 and Ppr1) showed that DNA-binding specificity is a function of a 19-amino-acid region C-terminal to the Zn–Cys cluster and not of the Zn–Cys cluster itself (Reece & Ptashne, 1993). Gal4 binding to multiple UAS_{GAL} elements is cooperative *in vitro* and leads to synergistic activation of transcription *in vivo* (Kang *et al*, 1993; Giniger & Ptashne, 1988). Gal4 has some ability to bind non-Gal4 UAS elements *in vivo* as shown by the activation of the proline utilization (PUT) pathway genes in a galactose-dependent manner in a *put3* background (D'Alessio & Brandriss, 2000).

In the absence of galactose, Gal4 is inactive owing to the binding of the repressor Gal80 to the Gal4-activation domain, which indicates that the interaction of this domain with the transcription machinery is prevented (Fig 1). For example, it has been shown that binding of Gal80 to Gal4 inhibits subsequent binding of the TATA-binding protein (TBP) or TFIIB *in vitro* (Wu *et al*, 1996) and that Gal80 blocks interactions of the Gal4-activation domain with Spt–Ada–Gcn5 acetyltransferase (SAGA) and NuA4 complexes (Carrozza *et al*, 2002). Relief of inhibition by Gal80 is dependent on a functional Gal3 protein. Biochemical studies have shown that Gal3 interacts with Gal80, and it is this interaction that is sensitive to the presence of galactose (Zenke *et al*, 1996). How exactly Gal3–Gal80 complex formation relieves Gal80 inhibition of Gal4 is not yet known. It was long believed that Gal3 interacts with Gal80 in the nucleus to elicit a conformational change in the Gal80–Gal4 complex (Leuther & Johnston, 1992). In favour of this model, recent studies using fluorescence resonance energy transfer (FRET) microscopy have confirmed that Gal4 and Gal80 stay associated in the presence of galactose (Bhaumik *et al*, 2004). However, localization studies show that Gal3 is exclusively a cytoplasmic factor. They also indicate that Gal80 dissociates from Gal4 on binding to Gal3 and 'shuttles' between the cytoplasm and the nucleus (Peng & Hopper, 2000). Artificial tethering of Gal3 in the cytoplasm does not preclude galactose-dependent activation of Gal4, and chromatin immunoprecipitation (ChIP) studies have shown that the association of Gal80 with the UAS_{GAL} is diminished when GAL gene expression is activated (Peng & Hopper, 2002).

Transcriptional activation by recruitment

Gal4 activates transcription by recruiting coactivators and the general transcription machinery to promoter regions through its activation domain (Fig 1). Important questions remain as to what are the functional targets of Gal4 and whether it contacts one or many proteins during the process of recruitment.

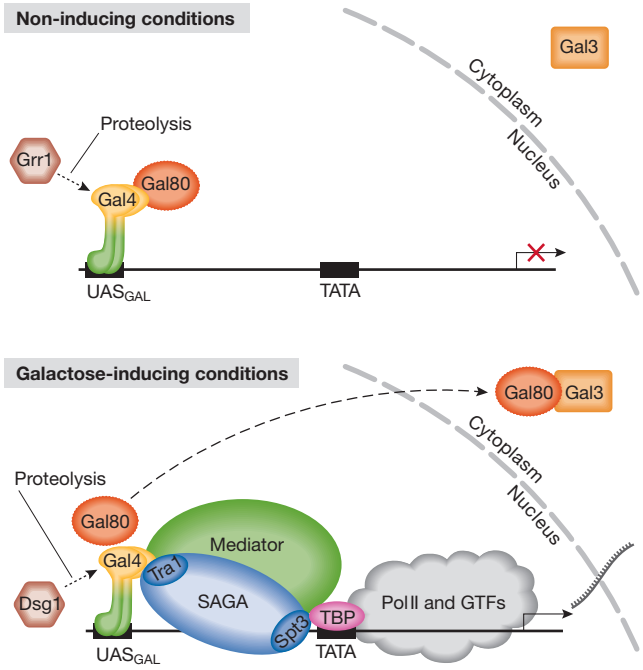

Fig 1 | Transcriptional activation by Gal4. Under non-inducing conditions, Gal4 activity is blocked by the interaction of Gal80 with the Gal4-activation domain. Gal4 levels are regulated by the F-box protein Grr1. On galactose induction, Gal80 is removed from the Gal4-activation domain, which is then able to recruit the transcriptional machinery. Dsg1 regulates turnover of transcriptionally active Gal4. GTFs, general transcription factors; SAGA, Spt-Ada-Gcn5 acetyltransferase; TBP, TATA-binding protein.

Proteins that have been shown to interact specifically with the Gal4-activation domain include TBP (Melcher & Johnston, 1995; Wu *et al*, 1996), TFIIB (Wu *et al*, 1996), Gal11 (a component of Mediator; Jeong *et al*, 2001), Cdk8 (also known as Srb10; Ansari *et al*, 2002), SWI/SNF (Yudkovsky *et al*, 1999), SAGA (Brown *et al*, 2001; Bhaumik *et al*, 2004), Srb4 (another component of Mediator; Koh *et al*, 1998) and proteasome components Sug1 (Gonzalez *et al*, 2002) and Sug2 (Chang *et al*, 2001).

The recent application of ChIP analyses and FRET microscopy to address the issues of factor occupancy and the kinetics of factor recruitment to the GAL genes have started to define the *in vivo* functions of the Gal4-activation domain. The conclusion of these studies is that SAGA is a physiologically relevant and direct target of Gal4. SAGA recruitment is dependent on the activation domain of Gal4 and on Spt20, which is a SAGA component essential to the integrity of the complex (Bhaumik & Green, 2001; Bryant & Ptashne, 2003; Larschan & Winston, 2001). Recruitment of SAGA to the UAS_{GAL} does not require additional factors other than Gal4, which suggests that it is a direct target of the Gal4-activation domain (Bhaumik *et al*, 2004; Larschan & Winston, 2001, 2005; Bryant & Ptashne, 2003). The histone acetyl transferase (HAT) component of SAGA, Gcn5, is not required for SAGA recruitment and pre-initiation complex (PIC) formation (Bhaumik & Green, 2001), therefore SAGA works as a scaffold that helps to assemble the PIC at the promoter, and not as a HAT. In the absence of Spt3—another SAGA subunit—SAGA recruitment is modestly

reduced, whereas PIC formation and transcription are significantly reduced (Bhaumik & Green, 2001; Larschan & Winston, 2001). This is most probably owing to the requirement of Spt3 for recruitment of TBP to the promoter of GAL genes (Larschan & Winston, 2001). As reported by Bhaumik and colleagues, FRET microscopy has revealed that the Tra1 subunit in the SAGA complex is the target of the Gal4-activation domain (Bhaumik *et al*, 2004). The Gal4–Tra1 interaction depends on the presence of both the activation and DNA-binding domains of Gal4, which indicates that Gal4 has to be bound to the UAS_{GAL} to interact with SAGA (Bhaumik *et al*, 2004). ChIP studies have shown that SAGA is the first complex to bind the GAL gene promoters on induction (Bryant & Ptashne, 2003; Bhaumik & Green, 2001), which supports the evidence that it is a direct target of Gal4.

Is SAGA the only direct target of the Gal4 activation domain, or can Gal4 target multiple coactivators during transcriptional activation? Recruitment of Mediator subunits to the UAS_{GAL} has been shown (Kuras *et al*, 2003; Bhaumik *et al*, 2004; Bryant & Ptashne, 2003; Lemieux & Gaudreau, 2004; Larschan & Winston, 2005). However, reports vary as to whether or not SAGA is required for Mediator recruitment. Green and co-workers have shown that SAGA is essential for Mediator recruitment to the UAS_{GAL}, and they were unable to detect an interaction between the Srb4 and Gal11 subunits of Mediator and Gal4 by FRET microscopy (Bhaumik *et al*, 2004). Conversely, three reports have shown that Mediator can be recruited to GAL genes independently of SAGA (Bryant & Ptashne, 2003; Larschan & Winston, 2005; Lemieux & Gaudreau, 2004). Results of recent *in vitro* cross-linking studies support the idea that Mediator might be bound directly by Gal4, as they have shown that both SAGA and Mediator interact directly with Gal4 and the unrelated acidic activator Gcn4 (Reeves & Hahn, 2005; Fishburn *et al*, 2005). However, some reports show that the recruitment of Mediator to the Gal4-dependent genes *in vivo* is diminished in the absence of SAGA (Larschan & Winston, 2005) or modestly delayed (Lemieux & Gaudreau, 2004), whereas others report that Mediator binds with similar kinetics in the absence of SAGA, but that this does not lead to productive PIC formation (Bryant & Ptashne, 2003). In conclusion, we believe that the data so far suggest that Mediator is probably a direct target of the activation domain of Gal4, but that the presence of SAGA at the UAS_{GAL} stabilizes the interactions of the Mediator subunits and is required for formation of the PIC and gene transcription.

It is possible that the Gal4-activation domain interacts directly with other transcription factors *in vivo*; however, such interactions, if they exist, must be weak and need to be stabilized by many contacts with several proteins or by a post-translational modification of Gal4, such as phosphorylation. The general transcription factors TBP and TFIIB bind to the Gal4-activation domain *in vitro* (Wu *et al*, 1996), but FRET microscopy failed to identify an *in vivo* interaction between these transcription factors and Gal4 in the presence of galactose (Bhaumik *et al*, 2004). Furthermore, recruitment of TBP to Gal4-dependent promoters requires factors such as Spt3, Ada1, Spt7 and Spt20 (all SAGA subunits, Srb9 and Srb10 (Srb8–Srb11 complex/Mediator), in addition to Gal4 (Dudley *et al*, 1999; Bhaumik & Green, 2001, 2002; Larschan & Winston, 2001, 2005). In the case of the Srb8–Srb11 complex, SAGA is required for binding of Srb9 to the UAS_{GAL} (Larschan & Winston, 2005). Similarly, ChIP studies suggest that Mediator, TAFs and RNA polymerase II are required for efficient recruitment of SWI/SNF to the *GAL1* promoter (Lemieux & Gaudreau, 2004).

Gal4 regulation by post-translational modification

Gal4 undergoes a series of phosphorylations, which produces three distinct migratory forms (a, b and c). Forms b and c are produced by the phosphorylation of form a. Form c is the slowest migrating of the three and is specific to Gal4, which is transcriptionally active in the presence of galactose, whereas form b exists even under non-inducing conditions (Sadowski *et al*, 1991; Muratani *et al*, 2005). Phosphorylation sites have been defined at Ser 691, Ser 696, Ser 699, and Ser 837 (Hirst *et al*, 1999; Sadowski *et al*, 1996). Form c corresponds to Gal4 phosphorylated at Ser 699 and Ser 837, whereas form b seems to contain only phospho-Ser 837 Gal4 (Sadowski *et al*, 1991; Muratani *et al*, 2005). The DNA-binding domain is also phosphorylated (Sadowski *et al*, 1996), but the exact residues have not been mapped. Phosphorylation of Gal4 occurs when the activator is engaged in activating transcription. It is executed by the RNA polymerase II-associated kinases Kin28(TFIIH) and Cdk8(Srb10) (Hirst *et al*, 1999) and requires other proteins in addition to Gal4, such as Mediator and SAGA subunits, general transcription factors and elongation factors (Muratani *et al*, 2005). The two kinases show different specificities *in vitro*: Kin28 predominantly phosphorylates Ser 837, whereas Srb10 phosphorylates Ser 699 (Hirst *et al*, 1999). How crucial is phosphorylation for Gal4 activity? The data so far suggest that phosphorylation regulates Gal4, but it is not absolutely essential for its activity. As shown by Ser to Ala mutagenesis, phosphorylation of residues other than Ser 699 seems to be irrelevant for activation of GAL genes (Rohde *et al*, 2000). Even phospho-Ser699 becomes dispensable when the concentration of galactose in the cell is high (Rohde *et al*, 2000), suggesting that this is a fine-tuning, rather than an on–off, mechanism. Furthermore, some mutations that severely impair the formation of Gal4 form c in galactose media, such as spt3Δ and bdf1Δ, have only a modest effect on transcriptional activation by Gal4 (Muratani *et al*, 2005).

The functional consequences of Gal4 phosphorylation are not fully understood, but some possibilities are emerging. Ser to Ala mutation at residue 699 affects induction of *GAL* gene transcription only when cells are *GAL80*$^+$ (Sadowski *et al*, 1996), suggesting that phospho-Ser 699 could have a role in stabilizing a Gal80–Gal4 conformation that is permissive to transcriptional activation. Another possible role for phospho-Ser 699 (and possibly phospho-Ser 837) could be to mark the Gal4 proteins that have already recruited the PIC to promoters, which would then target them for proteolysis with important consequences for productive versus unproductive expression of GAL genes (see below, Muratani *et al*, 2005).

Tansey and colleagues have recently uncovered a role for the ubiquitin degradation system in the turnover and activity of Gal4 (Muratani *et al*, 2005). Two systems operate in the regulation of Gal4 stability *in vivo*, through two separate F-box proteins Grr1 and Dsg1. Grr1 restricts the activity of Gal4 under non-inducing conditions (Fig 1) by degrading forms a and b of Gal4. Conversely, the Dsg1-dependent mechanism acts positively on the expression of GAL genes by turning over the transcriptionally active Gal4c (Fig 1). ChIP analyses have shown that Dsg1 is recruited to the UAS_{GAL} under inducing conditions. The role of Dsg1 is tied to the appropriate phosphorylation of the C-terminal domain of RNA polymerase II and the generation of translationally productive mRNA species. It is possible that phosphorylation of Gal4 could create a binding surface for Dsg1, which would in turn lead to ubiquitin-dependent proteolysis of Gal4c. The transition from initiation to elongation and subsequent post-transcriptional events that

produce translationally competent mRNAs (Muratani *et al*, 2005) are thus enabled. Phosphorylation, however, is probably not the only way by which Gal4c is recognized by Dsg1. In contrast to the deletion of DSG1, which completely abrogates Gal4 activity, the absence of Gal4c only modestly reduces Gal4-dependent transcription (Muratani *et al*, 2005). Furthermore, Dsg1 might have other targets in the transcription machinery that need to be degraded for Gal4-dependent transcription to be productive.

Conclusions

Transcriptional regulation of protein-encoding genes is a complex biochemical process that has yet to be fully explained. Studies on the Gal4-regulatory paradigm have greatly enhanced our understanding of transcriptional regulation in *S. cerevisiae* and have provided a conceptual framework for studies of transcriptional control in other organisms. As a detailed mechanistic understanding of transcription emerges, we can look forward to the application of this knowledge in the development of artificial transcriptional regulators or the development of drugs that modulate the transcriptional apparatus to target specific gene expression pathways *in vivo*.

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REFERENCES

Ansari AZ, Koh SS, Zaman Z, Bongards C, Lehming N, Young RA, Ptashne M (2002) Transcriptional activating regions target a cyclin-dependent kinase. *Proc Natl Acad Sci USA* **99:** 14706–14709

Bhaumik SR, Green MR (2001) SAGA is an essential *in vivo* target of the yeast acidic activator Gal4p. *Genes Dev* **15:** 1935–1945

Bhaumik SR, Green MR (2002) Differential requirement of SAGA components for recruitment of TATA-box-binding protein to promoters *in vivo*. *Mol Cell Biol* **22:** 7365–7371

Bhaumik SR, Raha T, Aiello DP, Green MR (2004) *In vivo* target of a transcriptional activator revealed by fluorescence resonance energy transfer. *Genes Dev* **18:** 333–343

Brown CE, Howe L, Sousa K, Alley SC, Carrozza MJ, Tan S, Workman JL (2001) Recruitment of HAT complexes by direct activator interactions with the ATM-related Tra1 subunit. *Science* **292:** 2333–2337

Bryant GO, Ptashne M (2003) Independent recruitment *in vivo* by Gal4 of two complexes required for transcription. *Mol Cell* **11:** 1301–1309

Carrozza MJ, John S, Sil AK, Hopper JE, Workman JL (2002) Gal80 confers specificity on HAT complex interactions with activators. *J Biol Chem* **277:** 24648–24652

Chang C, Gonzalez F, Rothermel B, Sun L, Johnston SA, Kodadek T (2001) The Gal4 activation domain binds Sug2 protein, a proteasome component, *in vivo* and *in vitro*. *J Biol Chem* **276:** 30956–30963

D'Alessio M, Brandriss MC (2000) Cross-pathway regulation in *Saccharomyces cerevisiae*: activation of the proline utilization pathway by Gal4p *in vivo*. *J Bacteriol* **182:** 3748–3753

Dudley AM, Rougeulle C, Winston F (1999) The Spt components of SAGA facilitate TBP binding to a promoter at a post-activator-binding step *in vivo*. *Genes Dev* **13:** 2940–2945

Fishburn J, Mohibullah N, Hahn S (2005) Function of a eukaryotic transcription activator during the transcription cycle. *Mol Cell* **18:** 369–378

Giniger E, Ptashne M (1988) Cooperative DNA binding of the yeast transcriptional activator *GAL4*. *Proc Natl Acad Sci USA* **85:** 382–386

Gonzalez F, Delahodde A, Kodadek T, Johnston SA (2002) Recruitment of a 19S proteasome subcomplex to an activated promoter. *Science* **296:** 548–550

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

Jeong C-J, Yang S-H, Xie Y, Zhang L, Johnston SA, Kodadek T (2001) Evidence that Gal11 protein is a target of the Gal4 activation domain in the mediator. *Biochemistry* **40:** 9421–9427

Johnston M, Dover J (1987) Mutations that inactivate a yeast transcriptional regulatory protein cluster in an evolutionarily conserved DNA binding domain. *Proc Natl Acad Sci USA* **84:** 2401–2405

Kang T, Martins T, Sadowski I (1993) Wild type GAL4 binds cooperatively to the *GAL1–10* UASG *in vitro*. *J Biol Chem* **268:** 9629–9635

Koh SS, Ansari AZ, Ptashne M, Young RA (1998) An activator target in the RNA polymerase II holoenzyme. *Mol Cell* **1:** 895–904

Kuras L, Borggrefe T, Kornberg RD (2003) Association of the Mediator complex with enhancers of active genes. *Proc Natl Acad Sci USA* **100:** 13887–13891

Larschan E, Winston F (2001) The *S. cerevisiae* SAGA complex functions *in vivo* as a coactivator for transcriptional activation by Gal4. *Genes Dev* **15:** 1946–1956

Larschan E, Winston F (2005) The *Saccharomyces cerevisiae* Srb8–Srb11 complex functions with the SAGA complex during Gal4-activated transcription. *Mol Cell Biol* **25:** 114–123

Lemieux K, Gaudreau L (2004) Targeting of Swi/Snf to the yeast GAL1 UASG requires the Mediator, TAFIIs, and RNA polymerase II. *EMBO J* **23:** 4040–4050

Leuther KK, Johnston SA (1992) Nondissociation of GAL4 and GAL80 *in vivo* after galactose induction. *Science* **256:** 1333–1335

Li Q, Johnston SA (2001) Are all DNA binding and transcription regulation by an activator physiologically relevant? *Mol Cell Biol* **21:** 2467–2474

Liang SD, Marmorstein R, Harrison SC, Ptashne M (1996) DNA sequence preferences of *GAL4* and *PPR1*: how a subset of Zn2 Cys6 binuclear cluster proteins recognizes DNA. *Mol Cell Biol* **16:** 3773–3780

Lohr D (1997) Nucleosome transactions on the promoters of the yeast GAL and PHO genes. *J Biol Chem* **272:** 26795–26798

Lohr D, Venkov P, Zlatanova J (1995) Transcriptional regulation in the yeast GAL gene family: a complex genetic network. *FASEB J* **9:** 777–787

Marmorstein R, Carey M, Ptashne M, Harrison SC (1992) DNA recognition by *GAL4*: structure of a protein–DNA complex. *Nature* **356:** 408–414

Melcher K, Johnston SA (1995) GAL4 interacts with TATA-binding protein and coactivators. *Mol Cell Biol* **15:** 2839–2848

Muratani M, Kung C, Shokat KM, Tansey WP (2005) The F box protein Dsg1/Mdm30 is a transcriptional coactivator that stimulates Gal4 turnover and cotranscriptional mRNA processing. *Cell* **120:** 887–899

Peng G, Hopper JE (2000) Evidence for Gal3p's cytoplasmic location and Gal80p's dual cytoplasmic–nuclear location implicates new mechanisms for controlling Gal4p activity in *Saccharomyces cerevisiae*. *Mol Cell Biol* **20:** 5140–5148

Peng G, Hopper JE (2002) Gene activation by interaction of an inhibitor with a cytoplasmic signaling protein. *Proc Natl Acad Sci USA* **99:** 8548–8553

Ptashne M, Gann A [Au: are these the editors?] (2002) *Genes & Signals*. Cold Spring Harbor, New York, USA: Cold Spring Harbor Laboratory Press

Reece RJ, Ptashne M (1993) Determinants of binding-site specificity among yeast C6 zinc cluster proteins. *Science* **261:** 909–911

Reeves WM, Hahn S (2005) Targets of the Gal4 transcription activator in functional transcription complexes. *Mol Cell Biol* **20:** 9092–9102

Ren B *et al* (2000) Genome-wide location and function of DNA binding proteins. *Science* **290:** 2306–2309

Rohde JR, Trinh J, Sadowski I (2000) Multiple signals regulate *GAL* transcription in yeast. *Mol Cell Biol* **20:** 3880–3886

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

Sadowski I, Costa C, Dhanawansa R (1996) Phosphorylation of Gal4p at a single C-terminal residue is necessary for galactose-inducible transcription. *Mol Cell Biol* **16:** 4879–4887

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

Yudkovsky N, Logie C, Hahn S, Peterson CL (1999) Recruitment of the SWI/SNF chromatin remodeling complex by transcriptional activators. *Genes Dev* **13:** 2369–2374

Zenke FT, Engels R, Vollenbroich V, Meyer J, Hollenberg CP, Breunig KD (1996) Activation of Gal4p by galactose-dependent interaction of galactokinase and Gal80p. *Science* **272:** 1662–1665