ACS chemical biology

LETTERS

pubs.acs.org/acschemicalbiology

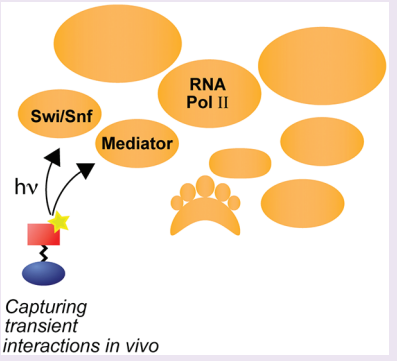
Caught in the Act: Covalent Cross-Linking Captures Activator−Coactivator Interactions *in Vivo*

Malathy Krishnamurthy,[†,∥] Amanda Dugan,[‡,∥] Adaora Nwokoye,[§] Yik-Hong Fung,[‡] Jody K. Lancia,[‡] Chinmay Y. Majmudar,[†] and Anna K Mapp[†,‡,§,*]

[†]Department of Chemistry, [‡]Program in Chemical Biology, and [§]Interdepartmental Program in Medicinal Chemistry, University of Michigan, Ann Arbor, Michigan 48109, United States

Supporting Information

ABSTRACT: Currently there are few methods suitable for the discovery and characterization of transient, moderate affinity protein−protein interactions in their native environment, despite their prominent role in a host of cellular functions including protein folding, signal transduction, and transcriptional activation. Here we demonstrate that a genetically encoded photoactivatable amino acid, *p*-benzoyl-L-phenylalanine, can be used to capture transient and/or low affinity binding partners in an *in vivo* setting. In this study, we focused on ensnaring the coactivator binding partners of the transcriptional activator VP16 in *S. cerevisiae*. The interactions between transcriptional activators and coactivators in eukaryotes are moderate in affinity and short-lived, and due in part to these characteristics, identification of the direct binding partners of activators *in vivo* has met with only limited success. We find through *in vivo* photo-cross-linking that VP16 contacts the Swi/Snf chromatin-remodeling complex through the ATPase Snf2(BRG1/BRM) and the subunit Snf5 with two distinct regions of the activation domain. An analogous experiment with Gal4 reveals that Snf2 is also a target of this activator. These results suggest that Snf2 may be a valuable target for small molecule probe discovery given the prominent role the Swi/Snf complex family plays in development and in disease. More significantly, the successful implementation of the *in vivo* cross-linking methodology in this setting demonstrates that it can be applied to the discovery and characterization of a broad range of transient and/or modest affinity protein−protein interactions.



Transient and moderate affinity protein−protein interactions (PPIs) play a critical role in the regulation of essential cellular processes including protein folding, ubiquitylation, and transcription. A number of disease states are believed to be the result of aberrations within these protein networks; therefore, a longstanding therapeutic goal has been to design small molecules that can tunably modulate the constituent interactions.[1−8] However, the discovery of small molecule modulators has been hindered by lack of structural and mechanistic information, in part due to the limitations of the approaches currently available for studying transient PPIs in their native environments. Techniques such as co-crystallization and co-purification *in vitro* and two-hybrid studies *in vivo* are best suited for probing stably associated proteins but are less ideal for studying proteins that engage in modest affinity and/or transient multiprotein binding interactions.[9−12] Here we demonstrate the *in vivo* covalent capture of such binding partners of the prototypical activator VP16, focusing on the chromatin-modifying coactivator complex Swi/Snf. Through these *in vivo* photo-cross-linking experiments we find that one region of VP16 contacts the Snf2 ATPase and a second relies upon the Snf5 scaffolding component for Swi/Snf binding, suggesting a cooperative recruitment mechanism for this complex at individual promoters. A similar *in vivo* analysis of the mechanistically related activator Gal4 reveals Snf2 to be a shared target, suggesting that the ATPase may be a viable target for small molecule intervention in the expanding roster of disease states that exhibit mis-regulated Swi/Snf.[13−15] The success of using a genetically encoded photoactivatable amino acid for characterizing activator−coactivator complexes *in vivo* indicates that this strategy can be implemented more broadly for the capture and discovery of transient protein−protein interactions in their native contexts.

Transcriptional activators are signal responsive regulatory proteins that assemble the transcriptional machinery at the promoter of a gene through dynamic binding interactions with a variety of coactivator complexes, including chromatin-modifying, helicase, and scaffolding complexes.[19,23] Activators are modular in architecture and are minimally composed of a DNA binding domain (DBD) that localizes the activator to its cognate DNA binding site and a transcriptional activation domain (TAD) that mediates the majority of contacts with transcriptional complexes. Although the interactions between activators and suppressor proteins can be high affinity and specific in nature, activator−coactivator interactions are mediated through lower affinity,

Received: August 21, 2011
Accepted: October 6, 2011
Published: October 06, 2011

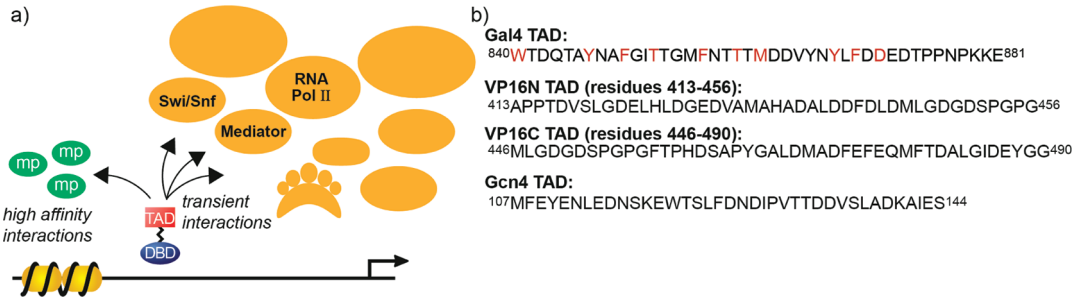


Figure 1. (a) The transcriptional activation domain (TAD) of amphipathic activators can engage in high affinity interactions, such as those with masking proteins (mp), but the interactions between the TAD and coactivator complexes are more moderate in affinity and transient in nature.[16−21] (b) Amphipathic activators share little sequence homology but do share binding targets, at least *in vitro*. The photo-cross-linking amino acid, Bpa, has been incorporated within the Gal4 TAD (positions of incorporation in red) with little impact on the function and binding profile of that TAD.[22]

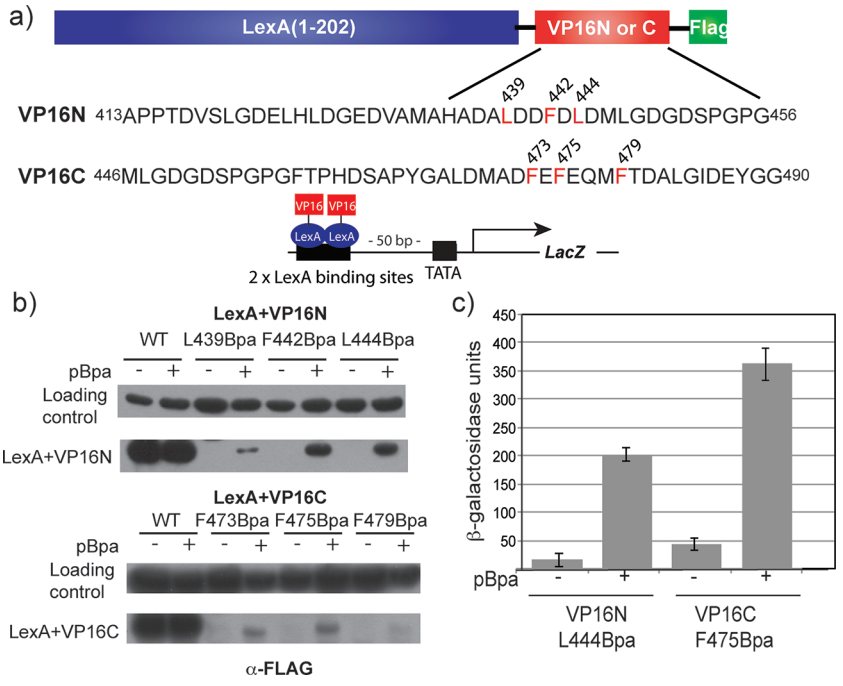


Figure 2. Incorporation of Bpa within the VP16 TAD. (a) Plasmids encoding the DNA binding domain (DBD) of LexA fused to either the N- or C-terminal VP16 TAD as well as a FLAG tag were constructed. The LexA DBD was utilized to exclusively examine transcriptional activation at the 2 unique LexA binding sites upstream of the LacZ reporter in *S. cerevisiae*. Positions at which Bpa mutagenesis was carried out are within regions of the VP16N or VP16C subdomains known to participate in coactivator binding (sites of incorporation highlighted in red). The loading control is a ∼71 kDa, FLAG-detected yeast protein whose expression level does not vary with activator identity.[22] (b) Yeast cells bearing plasmids encoding the various LexA+VP16 constructs and the Bpa specific tRNA/synthetase pair expressed by pSNRtRNA-pBpaRS were grown in the presence or absence of 1 mM Bpa and analyzed by Western blot. (c) LexA+VP16N L444Bpa and LexA+VP16C F475Bpa were assessed for their ability to upregulate transcription of an integrated LacZ reporter gene in *S. cerevisiae* as measured by liquid β-galactosidase assays. Each activity is the average of values from at least three independent experiments with the indicated error (SDOM). See Supporting Information Figure S7 for activity assays of the remaining mutants.

transient contacts (Figure1a).[16−21] *In vivo* co-localization studies have defined the complexes that are recruited by activators during transcription but have not readily provided information on the direct coactivator targets within these complexes.[24−26] For example, the well-characterized amphipathic activator VP16 has been shown to recruit the Swi/Snf chromatin-remodeling complex early in transcription initiation, as evidenced by both *in vivo* and *in vitro* co-localization studies.[27−32] *In vitro* assays have identified several subunits within this complex as possible targets of VP16, but *in vivo* interaction studies have not distinguished which of the components are the relevant binding partner(s).[17,33,34] Thus there is a clear need for *in vivo* methodologies that can capture transient activator−coactivator interactions in their native environment.

In vivo cross-linking with the genetically incorporated, photolabile amino acid *p*-benzoyl-L-phenylalanine (Bpa) has been demonstrated previously as a useful method for capturing direct, high affinity protein−protein interactions.[22,35−37] More specifically, Bpa placement within the TAD of the activator Gal4 did not impair function of the protein, and photoactivation led to covalent capture of its high affinity (low nanomolar K_D) suppressor protein Gal80.[22,38] However, although successful in the case of a very tight interaction, this method has not been

dx.doi.org/10.1021/cb200308e |*ACS Chem. Biol.* 2011, 6, 1321–1326

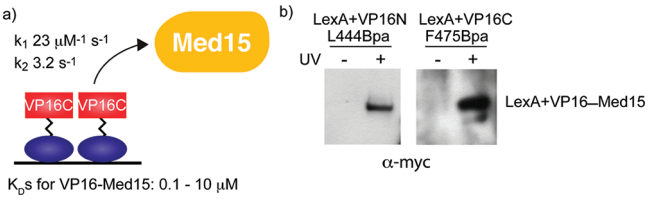


Figure 3. *In vivo* photo-cross-linking captures the moderate affinity interaction between LexA+VP16 and the Mediator protein, Med15. (a) VP16 has been shown to interact transiently with the coactivator Med15, as determined by measured kinetic rate constants. Equilibrium binding measurements place the affinity of the TAD for Med15 in the moderate category, with DNA-bound homodimers exhibiting the highest affinity (0.1 μM) and isolated TADs in the low to midmicromolar range.[16,39] (b) Live yeast cells bearing plasmids expressing LexA+VP16N L444Bpa or LexA+VP16C F475Bpa fusion proteins, in addition to a plasmid expressing myc-Med15(1-416) were irradiated with UV light (365 nm) for 30 min. Subsequently, cell lysates were immunoprecipitated with α-LexA and analyzed by Western blot (α-myc). For both constructs, a cross-link with Med15 is observed. Supporting Figure S1 shows expression of myc-Med15(1-416), and Supporting Figure S2 shows the full Western blot, complete with molecular weight references.

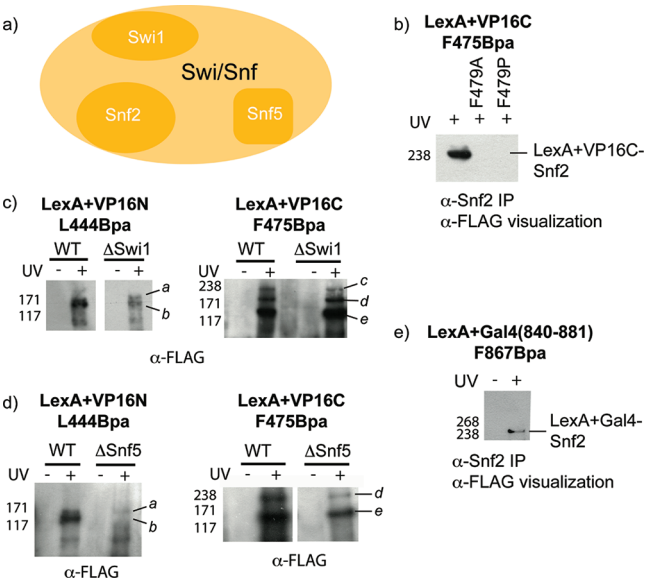


Figure 4. Analysis of VP16 cross-linking to the Swi/Snf coactivators, Snf2, Swi1, and Snf5. (a) The recruitment of the Swi/Snf chromatin remodeling complex by VP16 has been proposed to occur through interactions with the Snf2, Swi1, and Snf5 subunits, although the direct binding partners *in vivo* have not been determined.[17,33,34] (b) Live yeast cells expressing LexA+VP16C F475Bpa were irradiated with 365 nm light (30 min), and subsequently the cell lysates were immunoprecipitated with an antibody to Snf2 and resolved by Western blot (α-FLAG), revealing a direct interaction between VP16C and endogenous Snf2. In line with previous biochemical experiments, when phenylalanine 479 in VP16C was mutated to either alanine or proline, cross-linking to Snf2 was abolished. (c, d) LexA+VP16C F475Bpa and LexA+VP16N L444Bpa were expressed in yeast strains lacking either Swi1 or Snf5, and the live yeast cells were irradiated with 365 nm light. Subsequently, cell lysates were immunoprecipitated (α-LexA) and resolved by Western blot (α-FLAG). In the individual blots for LexA+VP16N, the marks *a* and *b* denote cross-linked protein bands at the appropriate size for Swi1 and Snf5, respectively. In the individual blots for LexA+VP16C, the marks *c*, *d*, and *e* indicate bands at the appropriate size for Snf2, Swi1, and Snf5, respectively. (e) To test if Gal4 also contacts Snf2, cross-linking experiments were carried out with live yeast cells expressing LexA+Gal4 F867Bpa as in panel b. The full Western blot of panels b and e can be found in Supplemental Figures S4 and S6, respectively.

employed in the case of moderate affinity transient interactions such as those between activators and coactivators. In this study, we test the utility of *in vivo* Bpa cross-linking for capturing VP16-coactivator interactions and for resolving the identity of the Swi/Snf components targeted by this activator.

Results and Discussion. The VP16 TAD is comprised of two subdomains that can function independently from one another, an amino terminal VP16N (residues 413−456) and a carboxy terminal VP16C (residues 446−490) (Figure 1b); therefore, we incorporated Bpa within regions of each subdomain shown to be involved in forming protein interactions. Further, because Bpa cross-linking is affected by its intrinsic reactivity and by positioning,[22,39] we examined six distinct mutants (VP16N: L439, F442, L444; VP16C: F473, F475, F479).[40−42] Bpa incorporation into VP16 is dependent on an enhanced nonsense suppression method that has been described previously.[22,43,44] Each Bpa-containing construct was expressed in *Saccharomyces cerevisiae* as a fusion protein bearing the bacterial LexA DBD and a carboxy-terminal FLAG tag for detection (Figure 2a). All six Bpa mutants were assayed for Bpa incorporation and activation potential in a yeast strain with an integrated LacZ reporter gene under the control of a GAL1 promoter bearing two LexA binding sites (Figure 2b,c). The LexA+VP16C F479Bpa mutant was poorly expressed and was therefore removed from further testing. The remaining mutants displayed good incorporation and activity profiles (Figure 2d and Supporting Information Figure S7).

A strong body of *in vitro* evidence exists to support the Mediator protein and coactivator Med15 as a target of VP16, and this model is supported by *in vivo* deletion and mutagenesis experiments.[16,39] The interaction between Med15 and activators such as VP16 is moderate in affinity, with dissociation constants ≥2 orders of magnitude weaker than that of the Gal4-Gal80 interaction (Figure 3a).[16,22,38,39] Thus, this appeared to be an excellent test case of the effectiveness of the *in vivo* cross-linking strategy for capturing moderate affinity binding interactions. We first tested the ability of each VP16 subdomain to cross-link to the coactivator Med15 *in vivo* by coexpressing myc-tagged Med15(1-416) alongside either LexA+VP16N L444Bpa or LexA+VP16C F475Bpa and irradiating live yeast with 365 nm UV light. The covalent adducts were isolated from the yeast lysate and analyzed by Western blot (Figure 3b). A direct contact between each subdomain of VP16 and Med15 was observed and was dependent upon irradiation, thus validating the utility of this method in capturing a moderate affinity, *in vivo* interaction of a transcriptional activator.

As outlined earlier, the Swi/Snf chromatin-modifying complex has also been proposed to be a direct binding partner of activators such as VP16, but there is conflicting evidence as to which subunit(s) serves as the activator-binding motif *in vivo*. VP16 enhances Swi/Snf recruitment to promoters such as the GAL1 promoter used in our studies and structural studies of Swi/Snf in complex with the nucleosome suggest that the catalytic subunit Snf2 is positioned close to the activator.[27−32,45−47] However, *in vitro* binding studies have shown several additional subunits can serve as binding partners (Swi1, Snf5).[17,33,34] We hypothesized that the *in vivo* cross-linking strategy could be used to test if the Swi/Snf complex is directly bound by VP16 in the cell and, if so,

dx.doi.org/10.1021/cb200308e |*ACS Chem. Biol.* 2011, 6, 1321–1326

to identify Swi/Snf subunits that are directly bound by VP16 in the native complex environment. In the case of both the LexA+VP16N L444Bpa and the LexA+VP16C F475Bpa activators, irradiation of the live yeast cells expressing the activators followed by visualization of all cross-linked products *via* immunodetection of the FLAG tag revealed several bands in the 130−220 kDa molecular weight range, consistent with the size range expected for covalent complexes with the Snf2, Swi1, and Snf5 subunits (Figure 4 and Supporting Information Figure S3). To test this, immunoprecipitation of whole-cell extracts from irradiated cells with an antibody to Snf2 was carried out. In these experiments, no detectable LexA+VP16N-Snf2 product was observed for any of the three LexA+VP16N Bpa mutants, but as seen in Figure 4b, the LexA+VP16C F475Bpa mutant cross-linked directly to Snf2. Consistent with this result, point mutations (F479A and F479P) known to decrease VP16 coactivator binding *in vitro* were introduced, and in line with these earlier biochemical experiments, abrogation of VP16 cross-linking to Snf2 *in vivo* was observed.[48−50] These results are consistent with the recent structural model proposed by Dechassa *et al.* that places Snf2 proximal to the transcriptional activator in the context of a Swi/Snf-nucleosome-activator complex.[32] Further, the data suggests that it is the C-terminus of the VP16 TAD that is responsible for the bulk of the Snf2 recruitment activity.[51]

In contrast to the Snf2 immunoprecipitation experiments, enrichment with either a Swi1 or Snf5 antibody did not result in any detectible cross-linked product.[52] To probe these interactions further, we generated yeast strains lacking either Swi1 or Snf5 and carried out cross-linking experiments. No differences in cross-linked product formation between the WT strain and Swi1 delete strain were observed with either VP16-derived activator, suggesting that Swi1 is not a direct target of VP16 (Figure 4c). In contrast, deletion of Snf5 disrupts the normal binding pattern of LexA+VP16N L444Bpa, consistent with Snf5 interacting with VP16N (Figure 4d). However, upon deletion of Snf5, LexA+VP16C F475Bpa displayed no change in binding pattern, suggesting that the VP16C TAD does not interact with Snf5. Together with the results of Figure 4b, these data support a model in which the subdomains of VP16 work cooperatively to recruit the Swi/Snf complex, with VP16C directly contacting Snf2 and VP16N depending on Snf5 during transcription initiation.

Snf2 is an ATPase that is essential for Swi/Snf function and is highly conserved among eukaryotes, making it a likely shared target among other transcriptional activators.[53] In fact, in addition to VP16, the amphipathic activators Gal4 and Gcn4 have been shown to recruit Swi/Snf to a variety of promoters *in vivo*, and *in vitro* binding studies have suggested that these activators contact a conserved set of targets within this complex.[17,31,33,34,54−58] To test if Snf2 is a shared target of these activators, Gal4 and Gcn4 were modified to contain pBpa within regions of each TAD implicated in coactivator binding and then tested for their ability to cross-link to Snf2. As shown in Figure 4e, Gal4 directly interacts with Snf2, whereas Gcn4 does not for any position tested (Supporting Information Figure S5). These data suggest that Snf2 (Brg1/Brm in metazoans) could be a key target for small molecule probe development in order to characterize the role of the conserved Swi/Snf complexes that are associated with pathophysiological processes.[13−15,59] However, further studies will be needed to dissect if VP16 and Gal4, as well as other activators, interact with the same binding site within Snf2. In addition, experiments with cross-linking moieties of distinct chemical reactivities will enable further refinement of the binding models.

Taken together, these data demonstrate that genetically encoded photo-cross-linkers are a viable and perhaps indispensible tool for capturing moderate affinity and transient protein−protein interactions *in vivo*. In this instance, employment of the *in vivo* photo-cross-linking strategy revealed an interaction model for the cooperative recruitment of the chromatin-modifying complex Swi/Snf to gene promoters and, further, identified the Snf2 coactivator and ATPase to be a direct target of the prototypical transcriptional activators VP16 and Gal4. These data represent a significant step toward the development of a complete interaction map of the direct binding partners of transcriptional activators, long an elusive goal. Successful implementation of the *in vivo* cross-linking methodology for this class of moderate affinity, transient interactions sets the stage for the dissection of the complex interactions of the many other cellular mechanisms that function through similar binding networks.

■ ASSOCIATED CONTENT

Supporting Information. This information is available free of charge *via* the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: amapp@umich.edu.

Author Contributions

‖These authors contributed equally to this work.

■ ACKNOWLEDGMENT

We are grateful to the NIH (GM 2RO106553) for support of this work. We thank Dr. L. Lee for construction of the Gcn4 expression plasmids and the Matzger laboratory for use of a photo-cross-linking apparatus.

■ REFERENCES

(1) Lee, L. W., and Mapp, A. K. (2010) Transcriptional switches: chemical approaches to gene regulation. *J. Biol. Chem. 285*, 11033–11038.

(2) Koehler, A. N. (2010) A complex task? Direct modulation of transcription factors with small molecules. *Curr. Opin. Chem. Biol. 14*, 331–340.

(3) Arkin, M. R., and Whitty, A. (2009) The road less traveled: modulating signal transduction enzymes by inhibiting their protein-protein interactions. *Curr. Opin. Chem. Biol. 13*, 284–290.

(4) Evans, C. G., Chang, L., and Gestwicki, J. E. (2010) Heat shock protein 70 (hsp70) as an emerging drug target. *J. Med. Chem. 53*, 4585–4602.

(5) Ong, D. S., and Kelly, J. W. (2011) Chemical and/or biological therapeutic strategies to ameliorate protein misfolding diseases. *Curr. Opin. Cell Biol. 23*, 231–238.

(6) Berg, T. (2008) Inhibition of transcription factors with small organic molecules. *Curr. Opin. Chem. Biol. 12*, 464–471.

(7) Arndt, H. D. (2006) Small molecule modulators of transcription. *Angew. Chem., Int. Ed. 45*, 4552–4560.

(8) Blazer, L. L., and Neubig, R. R. (2009) Small molecule protein-protein interaction inhibitors as CNS therapeutic agents: current progress and future hurdles. *Neuropsychopharmacology 34*, 126–141.

(9) Berggård, T., Linse, S., and James, P. (2007) Methods for the detection and analysis of protein-protein interactions. *Proteomics 7*, 2833–2842.

(10) Tanaka, Y., Bond, M. R., and Kohler, J. J. (2008) Photocross-linkers illuminate interactions in living cells. *Mol. Biosyst. 4*, 473–480.
(11) Melcher, K. (2004) New chemical crosslinking methods for the identification of transient protein-protein interactions with multiprotein complexes. *Curr. Protein Pept. Sci. 5*, 287–296.
(12) Perkins, J. R., Diboun, I., Dessailly, B. H., Lees, J. G., and Orengo, C. (2010) Transient protein-protein interactions: structural, functional, and network properties. *Structure 18*, 1233–1243.
(13) Wilson, B. G., and Roberts, C. W. (2011) SWI/SNF nucleosome remodellers and cancer. *Nat. Rev. Cancer 11*, 481–492.
(14) Hang, C. T., Yang, J., Han, P., Cheng, H. L., Shang, C., Ashley, E., Zhou, B., and Chang, C. P. (2010) Chromatin regulation by Brg1 underlies heart muscle development and disease. *Nature 466*, 62–67.
(15) Hargreaves, D. C., and Crabtree, G. R. (2011) ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. *Cell Res. 21*, 396–420.
(16) Wands, A. M., Wang, N., Lum, J. K., Hsieh, J., Fierke, C. A., and Mapp, A. K. (2011) Transient-state kinetic analysis of transcriptional activator.DNA complexes interacting with a key coactivator. *J. Biol. Chem. 286*, 16238–16245.
(17) Ferreira, M. E., Hermann, S., Prochasson, P., Workman, J. L., Berndt, K. D., and Wright, A. P. H. (2005) Mechanism of transcription factor recruitment by acidic activators. *J. Biol. Chem. 280*, 21779–21784.
(18) Fuxreiter, M., Tompa, P., Simon, I., Uversky, V. N., Hansen, J. C., and Asturias, F. J. (2008) Malleable machines take shape in eukaryotic transcriptional regulation. *Nat. Chem. Biol. 4*, 728–737.
(19) Mapp, A. K., and Ansari, A. Z. (2007) A TAD further: exogenous control of gene activation. *ACS Chem. Biol. 2*, 62–75.
(20) Herbig, E., Warfield, L., Fish, L., Fishburn, J., Knutson, B. a., Moorefield, B., Pacheco, D., and Hahn, S. (2010) Mechanism of Mediator recruitment by tandem Gcn4 activation domains and three Gal11 activator-binding domains. *Mol. Cell. Biol. 30*, 2376–2390.
(21) Melcher, K. (2000) The strength of acidic activation domains correlates with their affinity for both transcriptional and non-transcriptional proteins. *J. Mol. Biol. 301*, 1097–1112.
(22) Majmudar, C. Y., Lee, L. W., Lancia, J. K., Nwokoye, A., Wang, Q., Wands, A. M., Wang, L., and Mapp, A. K. (2009) Impact of nonnatural amino acid mutagenesis on the *in vivo* function and binding modes of a transcriptional activator. *J. Am. Chem. Soc. 131*, 14240–14242.
(23) Ptashne, M., Gann, A. (2001) *Genes & Signals*, Cold Spring Harbor Laboratory, New York.
(24) Bryant, G. O., and Ptashne, M. (2003) Independent recruitment *in vivo* by Gal4 of two complexes required for transcription. *Mol. Cell 11*, 1301–1309.
(25) Cosma, M. P., Tanaka, T., and Nasmyth, K. (1999) Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. *Cell 97*, 299–311.
(26) Hall, D. B., and Struhl, K. (2002) The VP16 activation domain interacts with multiple transcriptional components as determined by protein-protein cross-linking *in vivo*. *J. Biol. Chem. 277*, 46043–46050.
(27) Memedula, S., and Belmont, A. S. (2003) Sequential recruitment of HAT and SWI/SNF components to condensed chromatin by VP16. *Curr. Biol. 13*, 241–246.
(28) Yudkovsky, N., Logie, C., Hahn, S., and Peterson, C. L. (1999) Recruitment of the SWI/SNF chromatin remodeling complex by transcriptional activators. *Genes Dev. 13*, 2369–2374.
(29) Herrera, F. J., and Triezenberg, S. J. (2004) VP16-dependent association of chromatin-modifying coactivators and underrepresentation of histones at immediate-early gene promoters during herpes simplex virus infection. *J. Virol. 78*, 9689–9696.
(30) Hassan, A. H., Neely, K. E., and Workman, J. L. (2001) Histone acetyltransferase complexes stabilize SWI/SNF binding to promoter nucleosomes. *Cell 104*, 817–827.
(31) Neely, K. E., Hassan, A. H., Wallberg, A. E., Steger, D. J., Cairns, B. R., Wright, A. P., and Workman, J. L. (1999) Activation domain-mediated targeting of the SWI/SNF complex to promoters stimulates transcription from nucleosome arrays. *Mol. Cell 4*, 649–655.
(32) Dechassa, M. L., Zhang, B., Horowitz-Scherer, R., Persinger, J., Woodcock, C. L., Peterson, C. L., and Bartholomew, B. (2008) Architecture of the SWI/SNF-nucleosome complex. *Mol. Cell. Biol. 28*, 6010–6021.
(33) Prochasson, P., Neely, K. E., Hassan, A. H., Li, B., and Workman, J. L. (2003) Targeting activity is required for SWI/SNF function *in vivo* and is accomplished through two partially redundant activator-interaction domains. *Mol. Cell 12*, 983–990.
(34) Neely, K. E., Hassan, A. H., Brown, C. E., Howe, L., and Workman, J. L. (2002) Transcription activator interactions with multiple SWI/SNF subunits. *Mol. Cell. Biol. 22*, 1615–1625.
(35) Chin, J. W., and Schultz, P. G. (2002) *In vivo* photocrosslinking with unnatural amino acid mutagenesis. *ChemBioChem 3*, 1135–1137.
(36) Mohibullah, N., and Hahn, S. (2008) Site-specific cross-linking of TBP *in vivo* and *in vitro* reveals a direct functional interaction with the SAGA subunit Spt3. *Genes Dev. 22*, 2994–3006.
(37) Hino, N., Okazaki, Y., Kobayashi, T., and Hayashi, A. (2005) Protein photo-cross-linking in mammalian cells by site-specific incorporation of a photoreactive amino acid. *Nat. Methods 2*, 3–8.
(38) Thoden, J. B., Ryan, L. A., Reece, R. J., and Holden, H. M. (2008) The interaction between an acidic transcriptional activator and its inhibitor. The molecular basis of Gal4p recognition by Gal80p. *J. Biol. Chem. 283*, 30266–30272.
(39) Majmudar, C. Y., Wang, B., Lum, J. K., Hakansson, K., and Mapp, A. K. (2009) A high-resolution interaction map of three transcriptional activation domains with a key coactivator from photo-cross-linking and multiplexed mass spectrometry. *Angew. Chem., Int. Ed. 48*, 7021–7024.
(40) Triezenberg, S. J., Kingsbury, R. C., and McKnight, S. L. (1988) Functional dissection of VP16, the trans-activator of herpes simplex virus immediate early gene expression. *Genes Dev. 2*, 718–729.
(41) Regier, J. L., Shen, F., and Triezenberg, S. J. (1993) Pattern of aromatic and hydrophobic amino acids critical for one of two subdomains of the VP16 transcriptional activator. *Proc. Natl. Acad. Sci. U.S.A. 90*, 883–887.
(42) Consistent with other reports, expression of the full length VP16 TAD proved to be toxic in yeast. Therefore, we chose to use individual subdomains of the VP16 TAD which did not result in cell death.
(43) Chin, J. W., Cropp, T. A., Anderson, J. C., Mukherji, M., Zhang, Z., and Schultz, P. G. (2003) An expanded eukaryotic genetic code. *Science 301*, 964–967.
(44) Wang, Q., and Wang, L. (2008) New methods enabling efficient incorporation of unnatural amino acids in yeast. *J. Am. Chem. Soc. 130*, 6066–6067.
(45) Côté, J., Quinn, J., Workman, J. L., and Peterson, C. L. (1994) Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science 265*, 53–60.
(46) Lemieux, K., and Gaudreau, L. (2004) Targeting of Swi/Snf to the yeast GAL1 UAS G requires the Mediator, TAF IIs, and RNA polymerase II. *EMBO J. 23*, 4040–4050.
(47) Peterson, C. L., and Workman, J. L. (2000) Promoter targeting and chromatin remodeling by the SWI/SNF complex. *Curr. Opin. Genet. Dev. 10*, 187–192.
(48) Jonker, H. R., Wechselberger, R. W., Boelens, R., Folkers, G. E., and Kaptein, R. (2005) Structural properties of the promiscuous VP16 activation domain. *Biochemistry 44*, 827–839.
(49) Uesugi, M., Nyanguile, O., Lu, H., Levine, A. J., and Verdine, G. L. (1997) Induced alpha helix in the VP16 activation domain upon binding to a human TAF. *Science 277*, 1310–1313.
(50) Yang, F., DeBeaumont, R., Zhou, S., and Naar, A. M. (2004) The activator-recruited cofactor/Mediator coactivator subunit ARC92 is a functionally important target of the VP16 transcriptional activator. *Proc. Natl. Acad. Sci. U.S.A. 101*, 2339–2344.
(51) Although this hypothesis is supported by the lack of Snf2 cross-linking with three different VP16N Bpa mutants and by biochemical binding data from other laboratories,[34] an alternative hypothesis is that

none of the VP16N Bpa mutants in our study are properly positioned for Bpa cross-linking with Snf2.

(52) Using these antibodies for detection of endogenous Swi1 and Snf5, we have observed bands corresponding to the molecular weight of free Swi1 and Snf5, but no cross-linked complex was detected. It is possible that a covalent cross-link formed as a result of these experiments may be interfering with epitope recognition by the antibody, resulting in an inability to detect a cross-linked product. Moreover, one of the challenges associated with this effort is that the Swi/Snf complex is present at very low concentrations, about 100−500 copies per cell. Due to the low expression levels of these coactivators in combination with the instability of the activator−coactivator cross-linked products and, presumably, modest cross-linking yields, mass spectrometric identification of any of the activator cross-linked bands have not been successful. Sinz, A. (2010) Investigation of protein-protein interactions in living cells by chemical crosslinking and mass spectrometry. *Anal. Bioanal. Chem. 8*, 3433–3440. Soutourina, J., Wydau, S., Ambroise, Y., Boschiero, C., and Werner, M. (2011) Direct interaction of RNA polymerase II and mediator required for transcription *in vivo*. *Science 331*, 1451–1454.

(53) Laurent, B. C., Treich, I., and Carlson, M. (1993) The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation. *Genes Dev. 7*, 583–591.

(54) Ferreira, M. E., Prochasson, P., Berndt, K. D., Workman, J. L., and Wright, A. P. H. (2009) Activator-binding domains of the SWI/SNF chromatin remodeling complex characterized *in vitro* are required for its recruitment to promoters *in vivo*. *FEBS J. 276*, 2557–2565.

(55) Schwabish, M. A., and Struhl, K. (2007) The Swi/Snf complex is important for histone eviction during transcriptional activation and RNA polymerase II elongation *in vivo*. *Mol. Cell. Biol. 27*, 6987–6995.

(56) Bryant, G. O., Prabhu, V., Floer, M., Wang, X., Spagna, D., Schreiber, D., and Ptashne, M. (2008) Activator control of nucleosome occupancy in activation and repression of transcription. *PLoS Biol. 6*, 2928–2939.

(57) Govind, C. K., Yoon, S., Qiu, H., Govind, S., and Hinnebusch, A. G. (2005) Simultaneous recruitment of coactivators by Gcn4p stimulates multiple steps of transcription *in vivo*. *Mol. Cell. Biol. 25*, 5626–5638.

(58) Swanson, M. J., Qiu, H., Sumibcay, L., Krueger, A., Kim, S., Natarajan, K., Yoon, S., and Hinnebusch, A. G. (2003) A multiplicity of coactivators is required by Gcn4p at individual promoters *in vivo*. *Mol. Cell. Biol. 23*, 2800–2820.

(59) Reisman, D., Glaros, S., and Thompson, E. A. (2009) The SWI/SNF complex and cancer. *Oncogene 28*, 1653–1668.