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Tra1 as a screening target for transcriptional activation domain discovery

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We dedicate this to Professor Carlos Barbas III in honor of his being awarded the 2009 Tetrahedron Young Investigator Award

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ABSTRACT

There is tremendous interest in developing activator artificial transcription factors that functionally mimic endogenous transcriptional activators for use as mechanistic probes, as components of synthetic cell circuitry, and in transcription-targeted therapies. Here, we demonstrate that a phage display selection against the transcriptional activation domain binding motif of the coactivator Tra1(TRRAP) produces distinct sequences that function with similar binding modes and potency as natural activators. These findings set the stage for binding screens with small molecule libraries against TAD binding motifs to yield next-generation small molecule TADs.

The transcriptional mis-regulation patterns associated with human disease have motivated the development of functional replacements of endogenous malfunctioning transcription factors for selective targeting of particular disease states.[1,2] One class of such molecules are activator artificial transcription factors (activator ATFs), molecules designed to up-regulate the transcription of pre-selected target genes in a manner analogous to their natural counterparts. The synthetic approach for constructing activator ATFs is a straightforward one in which a DNA targeting entity (DNA binding domain or DBD) that localizes the ATF to a particular promoter is coupled to a transcriptional activation domain (TAD) that stimulates assembly of the transcriptional machinery at the promoter.[1,2] Among activator ATFs it is protein-based molecules that have seen the most advances towards therapeutic and biomanufacturing applications.[3–5] This success is based upon molecules with novel DNA binding domains (designer zinc fingers) coupled to natural TADs such as those derived from the viral protein VP16.[6–8] There are potential disadvantages to using natural/endogenous TADs for activator ATF construction such as interactions with the endogenous regulatory machinery (proteasome, for example). However, it has proven quite challenging to identify synthetic peptide and small molecule TADs that function well in cells for use in activator ATF construction.[1]

One of the difficulties associated with the discovery of effective TAD replacements is that natural TADs function by using a single peptide sequence to interact with multiple binding partners and these interactions are poorly characterized at the molecular level.[1,9–11] In the case of the most well-characterized eukaryotic activator Gal4, for example, biochemical and genetic evidence suggests that it stimulates assembly of the transcriptional machinery at a promoter during transcription initiation through direct binding interactions between its TAD and at least three distinct proteins residing in the Mediator and SAGA (chromatin remodeling) complexes.[10,12–15] Consistent with a multi-partner binding profile being critical for robust cellular function, peptide TADs obtained through a screen against the Mediator protein Med15 that function exclusively through Med15 binding display modest activity.[16–18] Further, the potent peptidic activator XL_Y originally thought to function exclusively through Med15 binding was subsequently found to require an additional binding partner.[19–21] Thus, the challenge for artificial TAD discovery is to develop an approach to identify peptides that interact with a similar array of binding partners using a single sequence.

Here we isolate the activator-binding module of the SAGA component Tra1 and identify ligands for this module using a phage display strategy. The sequences thus obtained are distinct from natural TADs yet interact with the same binding site(s). The Tra1 activator-binding motif appears to share significant similarities with other coactivators as these ligands also bind to the Mediator protein Med15. The results suggest that Tra1 is an excellent target for a small molecule screen since ligands that bind to this motif are also able to interact with other key transcriptional machinery proteins and function similar to endogenous activators.

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Tra1 is the yeast homolog of mammalian TRRAP and resides in the chromatin remodeling complex SAGA.[22] It is a large protein (437 kDa) containing a C-terminal PI3 K-like domain, as well as FAT and C-FAT domains.[23,24] In addition, several lines of evidence suggest that the C-terminal region of Tra1 (residues 1900-3744) are contacted by transcriptional activation domains of activators as part of transcription initiation.[25–27] Although it thus appeared likely that ligands for Tra1 would function as transcriptional activation domains, it was first necessary to define more precisely the region of the protein that contains the activator binding surface. Overlapping fragments that spanned residues 1905-3524 of Tra1 were generated and fluorescence polarization binding experiments with fluorescein-tagged TADs from Gal4, Gcn4 and VP16 (VP2) were carried out (Figs. 1 and 2a). It was observed that all three TADs interact with Tra1(3092-3524) with micromolar dissociation constants, with the TADs of Gal4 and VP2 binding more strongly than that of Gcn4 (Supplementary Fig. S1). Thus, this fragment of Tra1 was used for ligand selection in subsequent experiments.

In order to isolate ligands for Tra1(3092-3524), three rounds of positive phage display selection were performed using a dodecamer randomized peptide library (New England Biolabs). The selection was performed under two different conditions; Group A ligands were isolated in the presence of excess VP2 in order to bias against the selection of ligands that target the endogenous activator binding sites within this fragment of Tra1. For Group B ligands, the selection process was carried out without VP2 or any other biasing element. In both groups the ligands lack the preponderance of acidic amino acids that is often associated with amphipathic TADs such as Gal4 and Gcn4 that interact with Tra1. To evaluate their function, plasmids encoding each ligand fused to the Gal4 DBD were constructed and the activity assessed in β-galactosidase transcription assays in *S. cerevisiae*. Among these, only ligand B1 (ENSPLWWPQPLA) displayed significant activity, approximately 50-fold activation relative to the DNA binding domain alone, with several others stimulating to more modest levels (10-15-fold) (Fig. 2 and Supplementary Fig. S2). Because B1 was selected in the absence of competing VP2, we assessed if it interacts with the VP2 binding site within Tra1. Indeed, a competitive ELISA revealed that B1 is displaced from Tra1 by VP2 (Fig. 3). Thus the two TADs appear to target an overlapping binding site within Tra1, although a VP2-induced conformational change cannot be ruled out. A fluorescein-labeled version of ligand B1 was used to measure the affinity for Tra1, with a K_D of 92 ± 5 μM obtained (Supplementary Fig. S3). This is approximately threefold weaker than VP2 (33 ± 3 μM) but similar to that of Gcn4. The remainder of the experiments thus focused on ligand B1.

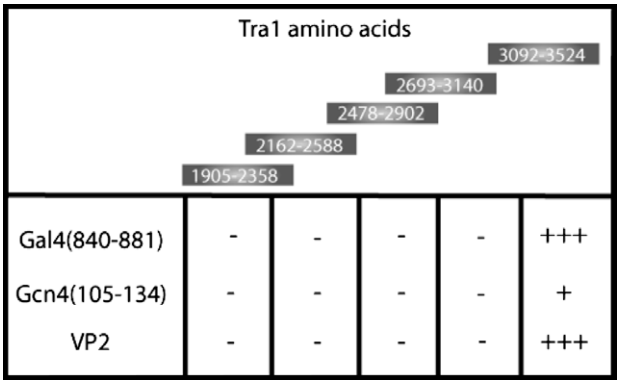

Figure 1. Identification of the TAD-interaction region of Tra1. Fluorescence polarization was used to determine dissociation constants of TADs with different fragments of Tra1 in vitro. +++ indicates a K_D of 10–50 μM, + 100–200 μM and - no detectable binding. See Supplementary data for additional details.

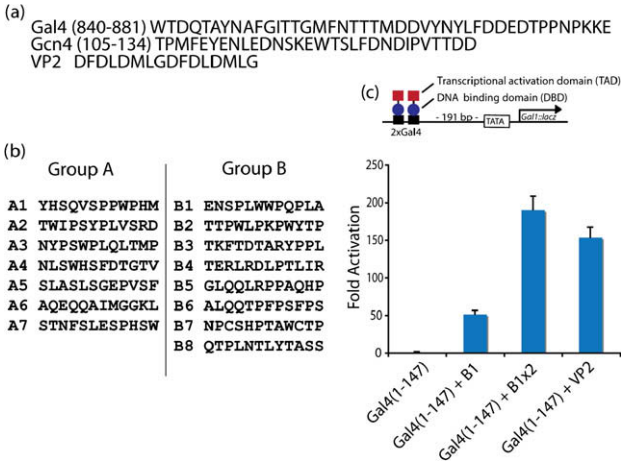

Figure 2. Ligands for Tra1. (a) Sequences of natural transcriptional activation domains that interact with Tra1. (b) Ligands isolated from phage display against Tra1. Group A ligands were isolated in the presence of a competitor TAD, VP2. ELISA was used to verify the binding of all selected sequences to Tra1 (data not shown). (c) Transcriptional activation of Tra1 ligands in yeast β-galactosidase assays as fusions to the Gal4(1-147) DBD. See Supplementary data for details.

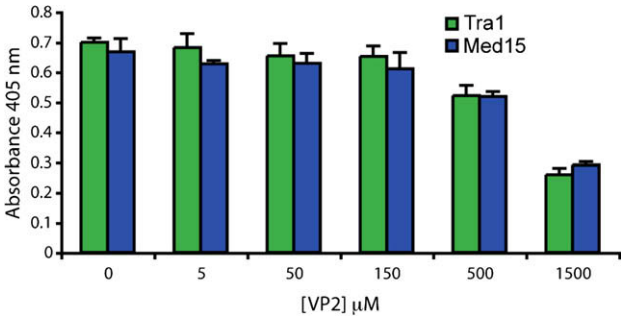

Figure 3. Competition between B1 and VP2. 10^{10} phage expressing B1 were incubated with varying amounts of VP2 and Med15(1-357) or Tra1(3092-3524) in a competitive ELISA. An anti-phage antibody was used to quantitate the amount of phage bound to Med15 or Tra1. See Supplementary data for additional details.

Amphipathic TADs such as VP2 typically show a synergistic enhancement in activity when their local concentration is increased at a promoter, either through increasing the number of DNA binding sites or by increasing the number of TAD units present within the activator.[28,29] Indeed, VP2 is actually a dimer of an eight residue amphipathic sequence. To see if B1 would exhibit similar characteristics, an activator in which the TAD is a dimer of B1 was constructed and tested in a β-galactosidase assay. As illustrated in Figure 2, this produced a synergistic (>95% confidence level) increase in activity, with approximately fourfold higher potency than the monomeric TAD. A dimer of B1 also binds more tightly to Tra1, exhibiting a K_D of 28 ± 1 μM, in line with that of VP2 (Supplementary Fig. S3).

As outlined earlier, another characteristic of amphipathic TADs is that they display a multi-partner binding profile that appears essential to their function.[11,30–32] In the case of Gcn4 and Gal4, for example, crosslinking experiments suggest that they contact both the Mediator (Med15) and SAGA (Tra1, Taf12) complexes as part of transcription initiation.[9,10] There is additional genetic and biochemical evidence consistent with Mediator and in particular Med15 being targeted by amphipathic activators.[14,33] We thus assessed if B1 would interact with the Med15 component of Mediator, again using fluorescence polarization. It was found that B1 interacts with Med15(1-357) with a 83 ± 5 μM K_D while the di-

mer binds with a 33 ± 2 μM K_D (Supplementary Fig. S3). Further, VP2 displaces Med15-bound B1 in a competitive ELISA, suggesting that as with Tra1, B1 and VP2 interact with the same binding site (Fig. 3). The role of the Med15 interaction in the function of B1 was assessed through transcriptional assays in yeast strains in which the relevant portions of Med15 had been deleted. It was found that deletion of Med15(2-345) results in a reduction (90%) in the transcriptional activity of B1 and 60% reduction of the B1 dimer (Supplementary Fig. S4). VP2 is similarly impacted by this deletion, with a 60% reduction in activity (Data not shown).

In summary, a binding selection against the chromatin modifying complex component and coactivator Tra1 produced ligands that mechanistically resemble natural amphipathic TADs despite differences in sequence composition. The most active of these ligands, B1, displays comparable in vivo activity to VP2. In vitro binding and in vivo mutagenesis also indicate that B1 binds to at least two of the sites targeted by VP2 in the transcriptional machinery, a site within Tra1 and also in the Mediator component Med15. Taken together, these data suggest that the activator binding surfaces in Tra1 and Med15 that VP2 and B1 interact bear significant similarities, despite little sequence or predicted structural homology between the two coactivators. Based on these results, screens or selections against other TAD binding motifs such as the KIX domain would be predicted to produce ligands that interact with multiple partners, particularly since the KIX motif has been identified in several coactivators.[18a–c,34,35] Finally, these data suggest that small molecule screens against the TAD binding motif of Tra1 and TRRAP should be an excellent starting point for the discovery of novel artificial TADs. Future detailed studies to discover and characterize the individual activator binding sites will further facilitate rationale design of synthetic TAD replacements.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.045.

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