Supporting Information for:

Tra1 as a screening target for transcriptional activation domain discovery

Chinmay Y. Majmudar, Anne E. Labut and Anna K. Mapp

Experimental

Protein Expression and peptide synthesis

Plasmids for bacterially expressing Tra1 fused to the maltose binding protein (MBP) were generated by inserting fragments of Tra1 amplified from yeast genomic DNA into pMal-c2g (New England Biolabs) using standard molecular biology techniques. Each fragment was expressed in Rosetta2(DE3) pLysS *E. coli* (Novagen) grown in Select APS Super broth (Difco) and induced with 0.1 mM IPTG at OD_{600} 0.5 for 12 h at 16 °C. The protein was lysed using sonication and purified using amylose resin (New England Biolabs). The protein concentration was measured using a Bradford assay (Bio-Rad) with BSA as the standard. The identity and purity of the fusion protein was verified by reducing SDS-PAGE with appropriate molecular weight standards. Med15(1-357) was expressed as previously described.[1]

Transcriptional activation domains VP2, Gal4(840-881) and Gcn4(105-134) were synthesized using solid phase synthesis and labeled with fluorescein. Dissociation constants were determined using fluorescence polarization-based binding experiments as previously described.[2]

Phage display

Phage display was performed using the Ph.D.-12 Phage Display Peptide Library Kit using the manufacturer recommended protocols (New England Biolabs). Briefly, 3 rounds of positive selection were performed against MBP-Tra1(3092-3524) in a NUNC maxisorp protein-binding 96 well plate. A negative selection against MBP was performed in between the first and second positive selection. For each positive selection, 200 μL 1 μM Tra1 in Storage buffer (10 mM PBS pH 7.0, 10% glycerol, 0.01% NP-40) was incubated in 2 wells of the plate at 4 °C. Following an overnight incubation, the protein solution was decanted off the plate. The plate was subsequently incubated with Blocking Buffer (10 mM PBS pH 7.0, 10% glycerol, 0.01% NP-40, 0.1% gelatin, 0.2% Tween-20, 10 mg/mL BSA and 0.02% NaN_3) for 2 h at 4 °C with shaking. After blocking, the wells were washed with Wash buffer (6 x 300 μL) (10 mM PBS, 0.1% Tween-20) and then 10 μL of the phage library in 100 μL of Binding buffer (10 mM PBS pH 7.0, 10% glycerol, 0.01% NP-40, 0.1% gelatin, 0.2% Tween-20, 10 mg/ml BSA) was added to each well. The phage were allowed to bind for 1 h at room temperature with shaking after which the wells were washed (10 x 300 μL) with Wash buffer. The phage were eluted by the addition of 150 μL Elution buffer (0.2 M glycine-HCl pH 2.2). After a 10 min incubation, 22.5 μL of Neutralizing buffer (1M Tris pH 9.0) was added. The eluted phage were amplified in ER2738 *E. coli* and precipitated using PEG/NaCl and used for subsequent pannings of selection. For the second and third round of selection, in one well of the 96-well plate, the Binding buffer used contained 500 μM VP2 while the other well contained Binding buffer without VP2. The third round of positive selection was performed similar

to the 2nd selection with the two different binding buffers except the stringency of the Wash buffer was increased to contain 0.5% Tween-20. To identify the peptide sequences encoded by each bound phage, individual phage isolated after the second and third rounds of positive selection were amplified to extract their DNA, which was sequenced at the University of Michigan Sequencing Core.

Competitive ELISA

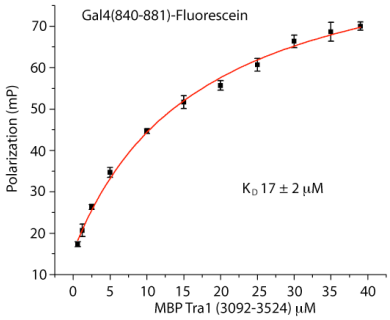
For the ELISA, 200 μL of 1 μM Tra1 or Med15 was added to each well of a NUNC maxisorp protein-binding 96 well plate and incubated overnight at 4 °C. The plate was blocked with Blocking buffer (10 mg/mL BSA, 10 mM PBS, 0.5% Tween-20) for 1 hr. Subsequently, 200 μL of 10^{10} phage was added to each well in 10 mM PBS, 0.5% Tween-20 with varying amounts of VP2 and incubated for 1 h. After 6 washes with 10 mM PBS, 0.5% Tween-20, 200 μL of anti-M13-hrp antibody (1:5000 dilution) was added in Blocking buffer to each well for 1 h. After 6 washes with 10 mM PBS, 0.5% Tween-20 the phage were detected using 200 μL of ABTS solution in sodium citrate with hydrogen peroxide as per the NEB phage display manual. The substrate was incubated for 1 h and the absorbance at 405 nm was measured using an absorbance plate reader (Tecan Genios Pro).

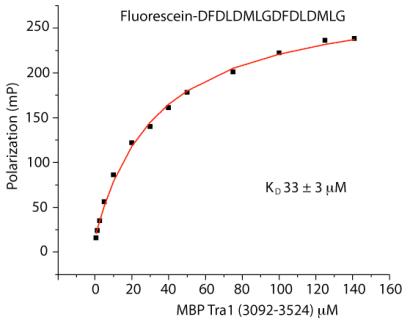
β-galactosidase assays

The function of ligands isolated from phage display was examined in yeast by evaluating plasmids encoding each ligand fused to the Gal4 DBD (pGBKT7, Clonetech) using quantitative liquid β-galactosidase assay in the yeast strain JPY52::JP188 as described previously.[3] The plasmid expressing the dimer of B1 fused to Gal4(1-147) was constructed by inserting a short linker (GSGGSG) in between the two B1 monomers.

References

1. DelProposto, J.; Majmudar, C. Y.; Smith, J. L.; Brown, W. C. *Protein Expr Purif* **2009**, *63*, 40-9.
2. Wu, Z.; Belanger, G.; Brennan, B. B.; Lum, J. K.; Minter, A. R.; Rowe, S. P.; Plachetka, A.; Majmudar, C. Y.; Mapp, A. K. *J Am Chem Soc* **2003**, *125*, 12390-1.
3. Majmudar, C. Y.; Lum, J. K.; Prasov, L.; Mapp, A. K. *Chem Biol* **2005**, *12*, 313-21.





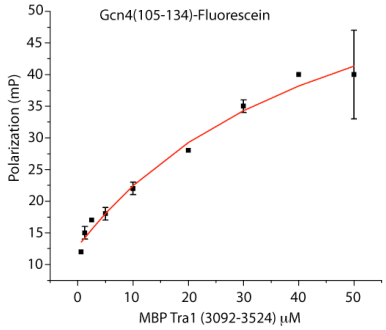


Figure S1. Binding experiments with natural TADs and Tra1(3092-3524). A constant 50 nM concentration of the fluorescein-labeled variant of each TAD was incubated with varying concentrations of MBP-Tra1(3092-3524) for ten minutes at room temperature and the resultant polarization values at each protein concentration were obtained on a Tecan Genios Pro plate reader. Each value is the average of three independent experiments with the indicated error (SDM).

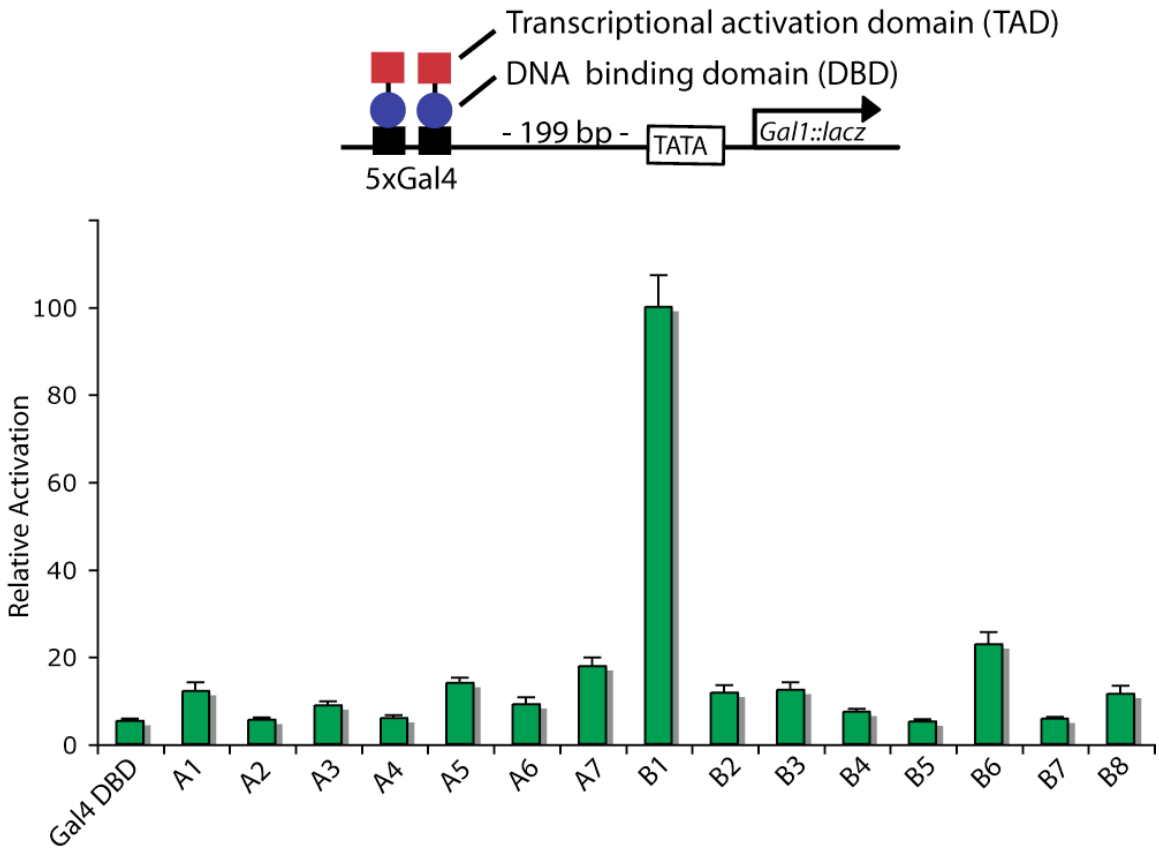


Figure S2. Activity of Tra1 ligands in yeast. β-galactosidase assays were performed in yeast strain LS41 to measure the activity of each ligand fused to the Gal4(1-147) DBD. The activity of the ligands is normalized to the activity of the most active ligand B1 for display purposes. Each value is an average of at least 3 independent experiments with the indicate error (SDOM). As discussed in the main text, B1 is the most active of the group with A1, A5, A7 and B8 exhibiting moderate activity. The differences in activity are in some cases due to attenuated binding to Tra1. For example, we were unable to measure dissociation constants for A7 and B6 in complex with Tra1. A second contributing factor to the differences in activity may be that some ligands may target binding sites that are not relevant for transcriptional activity; however, this has not been directly tested for these ligands.

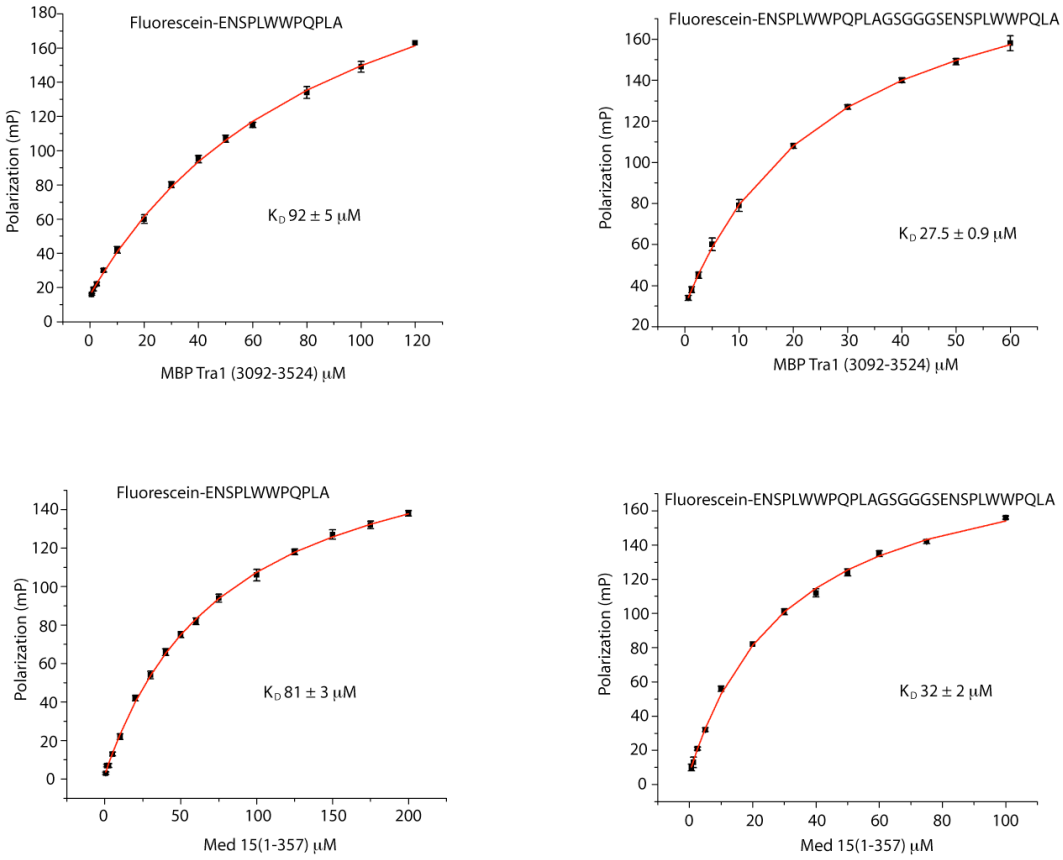


Figure S3. Dissociation constants for B1. A constant 50 nM concentration of fluorescein-labeled B1 was incubated with varying concentrations of MBP-Tra1(3092-3524) or Med15(1-357) for ten minutes at room temperature and the resultant polarization values at each protein concentration were obtained using a Tecan Genios Pro plate reader. Each value is the average of three independent experiments with the indicated error (SDM).

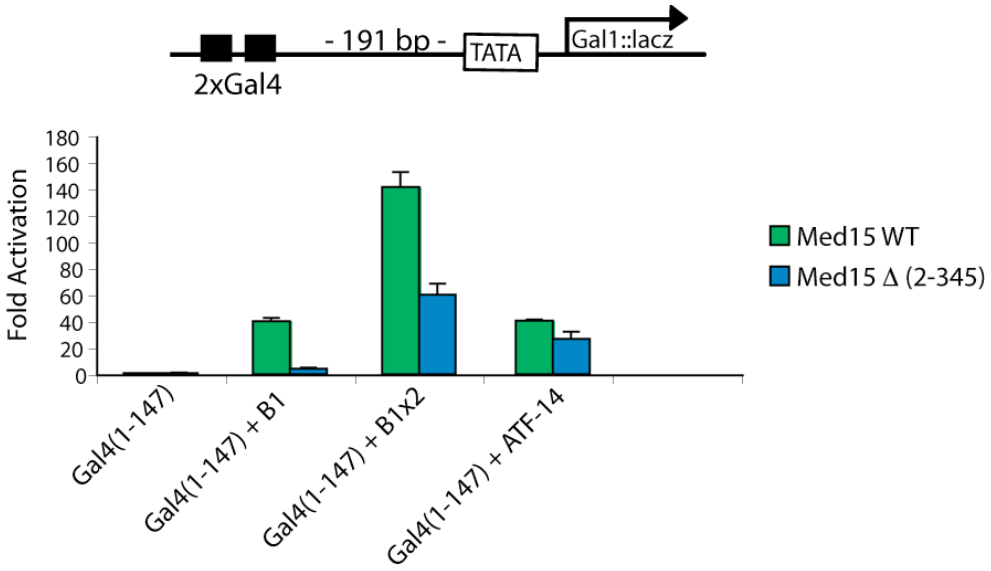


Figure S4. Med15 dependence of Tra1 ligands. β-galactosidase assays were performed in the Med15 delete yeast strain JPY52::JP188 transformed with a plasmid expressing full length Med15 or Med15 Δ(2-345) to measure the activity of each ligand fused to the Gal4(1-147) DBD. ATF-14 (CGSDALDDFDLDML) is a TAD derived from the endogenous activator VP16. Each value is an average of at least 3 independent experiments with the indicate error (SDOM).