Supporting Information

Mas et al. 10.1073/pnas.1017029108

SI Text

SI Experimental Procedures. ***Protein Expression and Purification.*** The carboxyl-terminal domain of the alpha subunit of the general transcription factor IIE (TFIIαCTD), the Pleckstin homology domain of Tfb1 (Tfb1PH), and the Pleckstrin homology domain of p62 (p62PH) were purified as previously described (1). The minimal transactivation domain of EKLF (EKLFTAD), EKLF-TAD1, EKLFTAD2 and CREB-binding protein (CBP)/p300 IBiD domain were expressed as GST-fusion proteins in *Escherichia coli* host strain TOPP2 (EKLFTAD1, EKLFTAD2, IBiD domain) or *E. coli* host strain Rosetta2 (EKLFTAD) and bound to glutathione resin. The resin-bound proteins were incubated 2 h with 100 units of thrombin (Calbiochem). Following the cleavage reaction, the proteins were dialyzed overnight into 5% aqueous acetic acid and further purified by reverse-phase HPLC over a C4 column (Vydac). The his-tagged KIX domain of CBP was first bound with chelating sepharose FF resin (GE Healthcare) charged with nickel, and the CBP-KIX protein was eluted with imidazole buffer [20 mM phosphate buffer-HCl (pH 7.4), 500 mM imidazol, 0.5 M NaCl]. The eluted protein was dialyzed overnight into 10% aqueous acetic acid and further purified by reverse-phase HPLC over a C4 column (Vydac).

The TAZ1 and TAZ2 domains of CBP were expressed in *E. coli* host strain BL21(DE3). The cells were grown at 37 °C, and protein expression was induced for 4 h with 0.7 mM IPTG at 37 °C. Cells were harvested, resuspended in buffer A [26 mM Tris buffer pH 7.4, 1 mM DTT], lysed by passage through a French press, and centrifuged at 15,000 × *g* for 20 min. The pellets from the centrifugation were then resuspended in buffer A with 6 M guanidinium HCl and centrifuged at 100,000 × *g* for 30 min. The supernatants were then dialyzed overnight into 5% aqueous acetic acid containing 2 mM DTT. The proteins were further purified by reverse-phase HPLC over a C4 column (Vydac). For NMR studies, uniformly (>98%) ^{15}N-labeled and $^{15}N/^{13}C$-labeled proteins were prepared in minimal media.

GAL4 Transactivation Assays in Yeast. Transactivation assays in yeast were performed as previously described (1). The results are presented as the mean of the percentage of the ß-galactosidase units of the tested LexA-fusion proteins on the ß-galactosidase units of the LexA-GAL4 positive control ± standard of the mean. Western-blot analyses were performed with an antibody directed at the LexA protein (Santa Cruz, sc-7544; 1:1,000 dilution) to verify equivalent expression of the LexA-fusion proteins.

NMR Samples. For structural studies of the Tfb1PH/EKLFTAD2 complex, the samples contained 1 mM ^{15}N- or 15N/^{13}C-labeled Tfb1PH and EKLFTAD2 was added to a final ratio of 1:2. These studies were performed in 20 mM sodium phosphate buffer pH 6.5, 1 mM EDTA, 1 mM DTT (NMR buffer) with either 90%H_2O/10%D_2O or 99.9% D_2O. For the labeled EKLF experiments, the samples contained 0.6 mM ^{15}N- or $^{15}N/^{13}C$-labeled EKLFTAD2, and Tfb1PH was added to a final ratio of 1:2 in NMR buffer.

For the NMR chemical shift titration studies of EKLFTADs with Tfb1PH, either unlabeled EKLFTAD1 or unlabeled EKLF-TAD2 was added to a sample containing 0.5 mM of ^{15}N-Tfb1PH in NMR buffer to a final ratio of 2:1. For the NMR chemical shift titration studies of EKLFTAD2 on p62PH, unlabeled EKLF-TAD2 was added to a final ratio of 1:1.5 to a sample containing 0.4 mM of ^{15}N-p62PH in NMR buffer.

For the NMR competition experiment, a sample containing 0.5 mM ^{15}N-labeled p53TAD2 (residues 40–73) in NMR buffer was used. To this sample, unlabeled Tfb1PH was added to a final concentration of 0.4 mM. In a second addition, unlabeled EKLF-TAD2 was added to a final concentration of 0.5 mM.

GAL4 Transactivation Assays in K562 Blood Cells. Luciferase assays in K562 blood cells were performed as described previously (2, 3). The minimal EKLFTAD (residues 2–106 of mouse EKLF) was fused to the DNA-binding domain of GAL4 (4) to create the GAL4-fusion proteins. The W73 are based on the numbering of the equivalent tryptophan residue in humans. Western-blot analysis with an antibody directed at the GAL4 protein is used to verify expression levels of the GAL-4 fusion protein.

1. Di Lello P, et al. (2008) p53 and TFIIEalpha share a common binding site on the Tfb1/p62 subunit of TFIIH. *Proc Natl Acad Sci USA* 105:106–111.
2. Sengupta T, Cohet N, Morle F, Bieker JJ (2009) Distinct modes of gene regulation by a cell-specific transcriptional activator. *Proc Natl Acad Sci USA* 106:4213–4218.
3. Chen XY, Bieker JJ (2004) Stage-specific repression by the EKLF transcriptional activator. *Mol Cell Biol* 24:10416–10424.
4. Chen X, Bieker JJ (1996) Erythroid Krueppel-like factor (EKLF) contains a multifunctional transcriptional activation domain important for inter- and intramolecular interactions. *EMBO J* 15:5888–5896.

LexA fusion protein	% β-Galactosidase units
LexA-EKLFTAD1	74.3 ± 8.7
LexA-EKLFTAD2	60.0 ± 7.0
LexA-p53TAD1	81.3 ± 9.0
LexA-p53TAD2	71.3 ± 3.5
LexA-GAL4TAD	100 ± 2
LexA	2 ± 1

Fig. S1. EKLFTAD1 and EKLFTAD2 function as independent activation domains in yeast. LexA-EKLF and LexA-p53 constructs were cotransformed into yeast with the reporter LexA operator-Lac-Z fusion plasmid pSH18-34. Results are presented as the mean of the percentages of the ß-galactosidase units of the tested fusion proteins on the ß-galactosidase units of the LexA-GAL4TAD positive control. Error bars represent standard error about the mean of three independent experiments.

Fig. S2. EKLFTAD2 binds to Tfb1PH. (*A*) Overlay of the two-dimensional ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) spectra for ^{15}N-labeled Tfb1PH in its free from (dark blue), in the presence of 0.5 equivalents (pink), 1 equivalent (green), and 2 equivalents (light blue) of EKLFTAD2. (*B*) Histogram of chemical shift variations ($\Delta\delta_{(\mathrm{ppm})} = [(0.17\Delta N_H)^2 + (\Delta H_N)^2]^{1/2}$ from titration in *A*. (*C*) Overlay of the 2D ^{1}H-^{15}N HSQC spectra for ^{15}N-labeled EKLFTAD2 in its free from (black), in the presence of 0. 5 equivalent (pink), and 1.5 equivalent (light blue) of Tfb1PH. Arrows highlight several signals that undergo significant changes in ^{1}H and ^{15}N chemical shifts in EKLFTAD2 upon formation of the TFb1PH/EKLFTAD2 complex. (*D*) Histogram of chemical shift variations ($\Delta\delta_{(\mathrm{ppm})} = [(0.17\Delta N_H)^2 + (\Delta H_N)^2]^{1/2}$ from titration in *C*.

Fig. S3. Electrostatic interactions in the Tfb1PH/EKLFTAD2 complex. Three-dimensional structure of Tfb1PH shown as a molecular surface in which the electrostatic potential is mapped between −10 kT (red) and +10 kT (blue) either in the presence (*A* and *C*) or absence of EKLFTAD2 (*B*). In *A*, the 3D structure of EKLFTAD2 is shown as mesh molecular surface with the same electrostatic potential mapped on the surface. In *C*, the two salt bridges that appear to contribute to the stability of the Tfb1PH/EKLFTAD2 complex are highlighted. One salt bridge involves Asp70 of EKLFTAD2 and Lys57 of Tfb1PH. The second salt bridge is between Asp76 of EKLFTAD2 and Lys101 of Tfb1PH. Asp70 and Asp76 of EKLFTAD2 are highlighted with red mesh and the backbone trace of EKLFTAD2 and the side chain of Trp73 are shown in yellow.

Fig. S4. Trp73 is crucial for the binding and in vivo activity of the minimal EKLFTAD. K562 cells were cotransfected with the 5xGAL-TK-β-reporter gene together with plasmids expressing the indicated GAL4-fusion proteins (4). A plasmid expressing *Renilla* luciferase was included as a control for normalization of transfection efficiency. The transcriptional activity of these proteins was normalized. Error bars represent standard error about the mean of multiple independent experiments. (Inset) The level of expression of the GAL4-fusion proteins as monitored by Western blot with an antibody against GAL4. Hsp90 levels are shown as a loading control.

Fig. S5. EKLFTAD2 and TFIIEα bind similarly to Tfb1PH/p62PH, but different from p53TAD2 and VP16C. (*A*) Overlay of EKLFTAD2 (yellow) and p53TAD2 (pink) shown as tubes on the surface of Tfb1PH (blue). EKLTAD2 is in an extended conformation and p53TAD2 forms a 9-residue α-helix. In the overlay, the side chains of Trp73 of EKLFTAD2 and Phe54 of p53TAD2 are located in the same pocket on Tfb1PH. (*B*) Overlay of EKLFTAD2 (yellow) and VP16 (orange) represented as tubes in complex with Tfb1PH (blue). EKLTAD2 is in an elongated form and VP16 forms a 9-residue α-helix and the side chains of Trp73 of EKLFTAD2 and Phe479 of VP16C are located in the same binding pocket on Tfb1PH. (*C*) Overlay of TFIIEαCTD and EKLFTAD2 on the 3D structure of Tfb1PH. EKLFTAD2 (yellow) and TFIIEαCTD (red) are represented as tubes on the surface of Tfb1PH (blue). In this overlay the side chains of Trp73 of EKLFTAD2 and Phe387 of TFIIEαCTD are highlighted to show that they are located in the same binding pocket on Tfb1PH/p62PH.

Fig. S6. EKLFTAD2 and TFIIEα bind similarly to Tfb1PH/p62PH. Overlay of TFIIEαCTD and EKLFTAD2 on the 3D structure of p62PH. EKLFTAD2 (yellow) and TFIIEαCTD (red) are represented as tubes on the surface of p62PH (aqua).

Table S1. List of intermolecular NOEs

Tfb1PH K47 HA	EKLF L77 HD
Tfb1PH Q49 HA	EKLF D76 HA
Tfb1PH Q49 HB	EKLF D76 HA
Tfb1PH Q49 HG	EKLF D76 HA
Tfb1PH A50 HB	EKLF L75 HD
Tfb1PH T51 HG	EKLF W73 HH2
Tfb1PH T51 HG	EKLF W73 HZ3
Tfb1PH S54 HA	EKLF A71 HB
Tfb1PH S54 HB	EKLF A71 HB
Tfb1PH S54 HB	EKLF A71 HA
Tfb1PH S55 HB	EKLF L75 HD
Tfb1PH K57 HE	EKLF A71 HA
Tfb1PH K57 HB	EKLF A71 HA
Tfb1PH M58 HE	EKLF G68 HA
Tfb1PH M59 HE	EKLF A69 HA
Tfb1PH M59 HE	EKLF D70 HB
Tfb1PH M59 HE	EKLF T72 HG
Tfb1PH M59 HE	EKLF T72 HB
Tfb1PH M59 HE	EKLF W73 HE3
Tfb1PH M59 HE	EKLF W73 HZ3
Tfb1PH M59 HE	EKLF W73HZ2
Tfb1PH M59 HE	EKLF W73 HH2
Tfb1PH M59 HB	EKLF W73HH2
Tfb1PH M59 HG	EKLF W73 HH2
Tfb1PH M59 HG	EKLF W73 HZ2
Tfb1PH M59 HE	EKLF L75 HD
Tfb1PH R61 HA	EKLF W73 HH2
Tfb1PH R61 HA	EKLF W73 HZ3
Tfb1PH M88 HA	EKLF W73 HH2
Tfb1PH M88 HA	EKLF W73 HZ3
Tfb1PH M88 HB	EKLF W73 HH2
Tfb1PH M88 HG	EKLF W73 HH2
Tfb1PH M88 HE	EKLF A69 HA
Tfb1PH M88 HE	EKLF A69 HB
Tfb1PH M88 HE	EKLF D70 HB
Tfb1PH M88 HE	EKLF A71 HB
Tfb1PH M88 HE	EKLF T72 HB
Tfb1PH M88 HE	EKLF W73 HE3
Tfb1PH M88 HE	EKLF W73 HZ2
Tfb1PH M88 HE	EKLF L75 HD
Tfb1PH K101 HE	EKLF L75 HG
Tfb1PH K101 HE	EKLF L75 HD
Tfb1PH Q105 HA	EKLF L79 HD
Tfb1PH Q105 HG	EKLF L79 HD
Tfb1PH Q105 HG	EKLF L79 HG
Tfb1PH Y111 HA	EKLF T80 HG
Tfb1PH K112 HE	EKLF L79 HD

Table S2. Structural statistics of the Tfb1PH/EKLFTAD2 complex

Restraints used for the structure calculations	
Total number of NOE distances restraints	1,686
Short-range (intraresidue)	675
Medium-range ($\|i - j\| \leq 4$)	575
Long-range	389
Intermolecular	47
Hydrogen bond	36
Number of dihedral angle restraints (ϕ, ψ)	116
Structural statistics	
rms deviations from idealized geometry	
Bonds, Å	0.0027 ± 0.00008
Angles, deg	0.4363 ± 0.0065
Impropers, deg	0.2871 ±0.0098
rms deviations from distance restraints, Å	0.0207 ± 0.0007
rms deviations from dihedral restraints, deg	0.5408 ± 0.0359
Ramachandran statistics, %*	
Residues in most favored regions	80.0
Residues in additional allowed regions	17.3
Residues in generously allowed regions	2.6
Residues in disallowed regions	0.1
Coordinate precision[†]	
Atomic pair wise rmsd, Å	
Tfb1PH/EKLFTAD2 complex	
Backbone atoms (C′, C^{α}, N)	0.70 ± 0.15
All heavy atoms	1.35 ± 0.19
Tfb1PH alone	
Backbone atoms (C′, C^{α}, N)	0.65 ± 0.15
All heavy atoms	1.32 ± 0.19
EKLFTAD2 alone	
Backbone atoms (C′, C^{α}, N)	0.68 ± 0.22
All heavy atoms	1.35 ± 0.41

The 20 conformers with the lowest energies were selected for statistical analysis. Because of the absence of medium-range, long-range, and intermolecular NOEs involving residues 51–58 and 86–90 of EKLFTAD2, these amino acids were not included in the calculations.

*Based on PROCHECK-NMR analysis.

[†]Only residues 4–63 and 86–114 of Tfb1PH and residues 70–79 of EKLFTAD2 were used for the rmsd calculations. Residues at the N terminus (1–3), at the C terminus (113–115), and in the flexible loop (64–85) of Tfb1PH, as well as residues at the N terminus (59–69) and at the C terminus (80–85) of EKLFTAD2, were not included in the calculation.