

Supplementary data for the manuscript

“14-3-3 (Bmh) proteins inhibit transcription activation by Adr1 through direct binding to its regulatory domain”

P. K. Parua, S. Ratnakumar, K. Braun, K.M. Dombek, E. Arms, P. Ryan, and E. T. Young

Supplementary Tables

Supplementary Table S1: Oligonucleotides

Name	Sequence	Target	Reference
KB2-GAL1.r1	ACGCTTAACTGCTCATTGCT	<i>GAL1-10p</i>	(Bryant and Ptashne, 2003)
KB3-GAL7.f1	TTTTTCCCCTTTATTTTGTTC	<i>GAL7p</i>	(Bryant and Ptashne, 2003)
KB4-GAL7.r1	GCTCAACAGTGCTCCGAAGT	<i>GAL7p</i>	(Bryant and Ptashne, 2003)
KB7-GAL2.f1	CCATGTGCCTTGGACGATAT	<i>GAL2p</i>	This study
KB8-GAL2.r1	CCATTTGCTCCCCATCTTGA	<i>GAL2p</i>	This study
ADH2.Q1	ACCATCCACTTCACGAGACTGA	<i>ADH2p</i>	(3)
ADH2.L	AAAAGTCGCTACTGGCACTC	<i>ADH2p</i>	(3)
tel.55	GCGTAACAAAGCCATAATGCCTCC	telomere	(3)
tel.56	CTCGTTAGGATCACGTTCTGAATCC	telomere	(3)
KB9-GAL1.f2	CGTTCATCAAGGCACCAAAT	<i>GAL1</i>	(Bryant and Ptashne, 2003)
KB10-GAL1.r2	TCAGAGGGCTAAGCATGTGT	<i>GAL1</i>	(Bryant and Ptashne, 2003)
KB11-GAL10.f1	ACGGAGATTATGGTGCCTTC	<i>GAL10</i>	(Bryant and Ptashne, 2003)
KB12-GAL10.r1	AATCAAACCTGGGGATTTTTGG	<i>GAL10</i>	(Bryant and Ptashne, 2003)
KB13-GAL7.f2	GGACCTCGCCTCGATTTTA	<i>GAL7</i>	(Bryant and Ptashne, 2003)
KB14-GAL7.r2	AATTCATCACCAGTCGCATTC	<i>GAL7</i>	(Bryant and Ptashne, 2003)

			2003)
KB15-ACT1.f2	TTCCCAGGTATTGCCGAAAG	<i>ACT1</i>	This study
KB16-ACT1.r2	CCAATCCAGACGGAGTACTTTC	<i>ACT1</i>	This study
KB18-GAL2.r2	CAACCAAACAGCCCATGAAG	<i>GAL2</i>	This study
KB19.GAL10p.f 2	GCTCGGCGGCTTCTAATC	pHZ18' (UASg- <i>CYC1p</i>)	This study
KB20.CYC1p.r1	TGCTGCAAAGGTCCTAATGTATAAG	pHZ18' (UASg- <i>CYC1p</i>)	This study
ADR1-1F	CCAAAAAAGAGATCGAATTCCAGAT GGCTAACGTAGAAAAACC	<i>ADR1</i>	This study
ADR1-20F	CCAAAAAAGAGATCGAATTCCAGTC GTGCTTTTCTAACGGCTT	<i>ADR1</i>	This study
ADR1-148F	CCAAAAAAGAGATCGAATTCCAGAT CAGGCATGCTCAAAAAT	<i>ADR1</i>	This study
ADR1-154F	CCAAAAAAGAGATCGAATTCCAGAT CCATAGTGGTAATTTAGG	<i>ADR1</i>	This study
ADR1-468F	CCAAAAAAGAGATCGAATTCCAGTC CCATTTGAACCTATCAA	<i>ADR1</i>	This study
ADR1-160R	CGACGGATCCCCGGGAATTGCCATG TCACCCTAAATTACCACTATGGA	<i>ADR1</i>	This study
ADR1-220R	CGACGGATCCCCGGGAATTGCCATG TCACTTACGTTTAAACATTAGCTT	<i>ADR1</i>	This study
ADR1-304R	CGACGGATCCCCGGGAATTGCCATG TCATATAATCCAAATTCATTGTT	<i>ADR1</i>	This study
ADR1-424R	CGACGGATCCCCGGGAATTGCCATG TCAATCGACGAAATCAGGTTGGT	<i>ADR1</i>	This study
ADR1-1323R	CGACGGATCCCCGGGAATTGCCATG TCAACTGTTTCCCTTTAGATGAT	<i>ADR1</i>	This study
ADR1-215F	CCAAAAAAGAGATCGAATTCCAGGC TAATGTTAAACGTAAGTACTTG	<i>ADR1</i>	This study
ADR1-255F	CCAAAAAAGAGATCGAATTCCAGGT	<i>ADR1</i>	This study

	TAAATTTTCTACGCCTGAATTA		
ADR1-280F	CCAAAAAAGAGATCGAATTCCAGAT GAATCTAGATTTAAACCTAAAT	<i>ADR1</i>	This study
ADR1-355F	CCAAAAAAGAGATCGAATTCCAGAA AGCCTATAATGATCATTGTTT	<i>ADR1</i>	This study
ADR1-305F	CCAAAAAAGAGATCGAATTCCAGAC AATGAATTTGGATTATAAATTG	<i>ADR1</i>	This study
ADR1-260R	CGACGGATCCCCGGGAATTGCCATG TCAAGGCGTAGAAAATTTAACAC	<i>ADR1</i>	This study
ADR1-310R	CGACGGATCCCCGGGAATTGCCATG TCAATAATCCAAATTCATTGTTG	<i>ADR1</i>	This study
ADR1-360R	CGACGGATCCCCGGGAATTGCCATG TCAATGATCATTATAGGCTTTTA	<i>ADR1</i>	This study
ADR1-420R	CGACGGATCCCCGGGAATTGCCATG TCAAGGTTGGTTGTCTGATACCT	<i>ADR1</i>	This study
ADR1-240F	CCAAAAAAGAGATCGAATTCCAGTT GCCCGACCAATCTTCGCT	<i>ADR1</i>	This study
ADR1-280R	CGACGGATCCCCGGGAATTGCCATG TCACATATTCAGGTCAAACGAAG	<i>ADR1</i>	This study
ADR1-300R	CGACGGATCCCCGGGAATTGCCATG TCAATCAGAACGGTTTAATGCTA	<i>ADR1</i>	This study

Supplementary Table S2. Plasmids.

Yeast plasmids			
pHZ18'	<i>URA3</i> -2 μ	UASGAL10-TATACYC1- <i>lacZ</i>	(32)
pGAD-C1	<i>LEU2</i> -2 μ	<i>ADH1p-GAD</i>	(42)
pOBD2	<i>TRP1</i> -CEN3	<i>ADH1p-GBD</i>	(67)
pKD134	<i>LEU2</i> -2 μ	<i>ADH1p-GBD-BMH1</i>	(25)
pKD8	<i>TRP1</i> -CEN3	empty vector	(Beier and Young, 1982)
pKD16	<i>TRP1</i> -CEN3	<i>ADR1</i>	(Dombek and Young, 1997)
pKD16HA	<i>TRP1</i> -CEN3	<i>ADR1-HA3</i>	This study
pKD14	<i>TRP1</i> -CEN3	<i>ADR1-S230A</i>	(Dombek et al., 1993)
pKD14HA	<i>TRP1</i> -CEN3	<i>ADR1-S230A-HA3</i>	This study
pKD17	<i>TRP1</i> -2 μ	<i>ADH1p-ADR1</i>	(Dombek and Young, 1997)
pKD17HA	<i>TRP1</i> -2 μ	<i>ADH1p-ADR1-HA3</i>	(53)
pGBDA1	<i>TRP1</i> -CEN4	<i>ADR1 (1-160)</i>	This study
pGBDA2	<i>TRP1</i> -CEN4	<i>ADR1 (20-220)</i>	This study
pGBDA3	<i>TRP1</i> -CEN4	<i>ADR1 (148-304)</i>	This study
pGBDA3SA	<i>TRP1</i> -CEN4	<i>ADR1 (148-304;S230A)</i>	This study
pGBDA4	<i>TRP1</i> -CEN4	<i>ADR1 (154-424)</i>	This study
pGBDA4SA	<i>TRP1</i> -CEN4	<i>ADR1 (154-424;S230A)</i>	This study
pGBDA5/6	<i>TRP1</i> -CEN4	<i>ADR1 (468-1323)</i>	This study
pGBDA11	<i>TRP1</i> -CEN4	<i>ADR1 (215-420)</i>	This study
pGBDA12	<i>TRP1</i> -CEN4	<i>ADR1 (215-360)</i>	This study
pGBDA13	<i>TRP1</i> -CEN4	<i>ADR1 (215-310)</i>	This study
pGBDA14	<i>TRP1</i> -CEN4	<i>ADR1 (215-260)</i>	This study
pGBDA15	<i>TRP1</i> -CEN4	<i>ADR1 (255-420)</i>	This study
pGBDA16	<i>TRP1</i> -CEN4	<i>ADR1 (255-360)</i>	This study
pGBDA17	<i>TRP1</i> -CEN4	<i>ADR1 (255-310)</i>	This study
pGBDA18	<i>TRP1</i> -CEN4	<i>ADR1 (280-310)</i>	This study
pGBDA19	<i>TRP1</i> -CEN4	<i>ADR1 (280-360)</i>	This study
pGBDA20	<i>TRP1</i> -CEN4	<i>ADR1 (280-420)</i>	This study
pGBDA21	<i>TRP1</i> -CEN4	<i>ADR1 (305-360)</i>	This study
pGBDA22	<i>TRP1</i> -CEN4	<i>ADR1 (305-420)</i>	This study
pGBDA23	<i>TRP1</i> -CEN4	<i>ADR1 (355-420)</i>	This study
pGBDA24	<i>TRP1</i> -CEN4	<i>ADR1 (148-260)</i>	This study
pGBDA25	<i>TRP1</i> -CEN4	<i>ADR1 (154-260)</i>	This study
pGBDA26	<i>TRP1</i> -CEN4	<i>ADR1 (241-280)</i>	This study
pGBDA27	<i>TRP1</i> -CEN4	<i>ADR1 (241-300)</i>	This study
<i>E. coli</i> plasmids			
pSZ21	Amp ^R	<i>BMH1</i> in pGEX-3X (<i>GST-BMH1</i>)	(Bertram et al., 1998)
pSZ22	Amp ^R	<i>BMH2</i> in pGEX-3X (<i>GST-BMH2</i>)	(Bertram et al., 1998)

Supplementary Table S3. Sequence identities in Adr1 orthologs of Ascomycetes.

Gene	Species	Adr1 orthologs		
		SeqLen	% Identity	% Similarity
Adr1	<i>S. cerevisiae</i>	1323	100.0	100.0
516.1	<i>S. bayanus</i>	1319	79.5	87.5
E09922g	<i>K. thermotolerans</i>	1119	37.7	52.4
47.17927	<i>K. waltii</i>	1003	34.5	50.3
H12958g	<i>S. kluyveri</i>	1129	36.8	52.2
529.13	<i>K. polysporus</i>	1410	32.3	47.8
A09416g	<i>Z. rouxii</i>	1346	32.8	47.0
AGR172W	<i>A. gossypii</i>	1057	39.0	56.0
721.92	<i>S. castellii</i>	1152	29.3	43.2
E04884g	<i>C. glabrata</i>	1489	28.1	41.4
F13046g	<i>K. lactis</i>	1379	24.7	39.2
Mxr1	<i>P. pastoris</i>	1155	21.8	35.7

Rsf2 orthologs				
Rsf2	<i>S. cerevisiae</i>	1380	100.0	100.0
YML081W	<i>S. cerevisiae</i>	1251	38.9	56.8
643.2	<i>S. bayanus</i>	1267	37.9	55.3
0B14894g	<i>Z. rouxii</i>	1119	43.1	62.2
B04477g	<i>K. lactis</i>	1332	37.4	52.3
AER159C	<i>A. gossypii</i>	1191	39.1	55.6
D18062g	<i>K. thermotolerans</i>	1128	42.1	59.5
47.16621	<i>K. waltii</i>	1178	41.0	58.4
G18062g	<i>S. kluyveri</i>	1238	40.6	56.9
H04213g	<i>C. glabrata</i>	1321	36.0	53.7
703.23	<i>S. castellii</i>	1341	35.3	52.7
1001.1	<i>K. polysporus</i>	1341	33.2	49.3
719.68	<i>S. castellii</i>	1350	36.4	51.3

Bryant, G.O. and Ptashne, M. (2003). Mol. Cell 11, 1301-1309.

Dombek, K. M., S. Camier, and E. T. Young. 1993. ADH2 expression is repressed by REG1 independently of mutations that alter the phosphorylation of the yeast transcription factor ADR1. Mol Cell Biol 13:4391-9.

Dombek, K. M., and E. T. Young. 1997. Cyclic AMP-dependent protein kinase inhibits ADH2 expression in part by decreasing expression of the transcription factor gene ADR1. Mol Cell Biol 17:1450-8.

Beier, D. R., and E. T. Young. 1982. Characterization of a regulatory region upstream of the ADR2 locus of *S. cerevisiae*. Nature 300:724-8.

Bertram, PG., Zeng, C., Thorson, J., Shaw, AS. and Zheng, XFS. (1998). The 14-3-3 proteins positively regulate rapamycin-sensitive signaling. Current Biology. 8: 1259.