SUPPLEMENTAL DATA

Forced homodimerization of the Fos leucine zipper in designed bHLHZ-like hybrid proteins MaxbHLH-Fos and ArntbHLH-Fos

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Table S1. Oligonucleotides Used in this Study*

No	Sequence	Note
1	TGCGGGGAACTGGCAACACACAAGCTGAAACTGACCAA	TFos fragment assembly
2	CAACAAGTTAGCAATTTCGGTTTGCAAAGCAGACTTTTCGTCTTCCAATTGGTCAGTTTC	TFos fragment assembly
3	GAAATTGCTAACTTGTTGAAGGAAAAGGAAAAGTTGGAGTTTATCTTGGCTGCTCACAGA	TFos fragment assembly
4	AAA**GGATCC**TGGTCTGTGAGCAGCCAAGAT	TFos fragment assembly
5	ACT**GAATTC**AGCTCTGCGGATAAAGAGAG	ArntbHLH$_{82-149}$ assembly
6	ATA**CTCGAG**GGATGTGTTGCCAGTTCCCC	ArntbHLH$_{82-149}$ assembly
7	CTAT**GAATTC**GCTGACAAGAGAGCTCACCACAACG	Max construct forward primer
8	ACAAGGCTACCGAATACATCCAATACATGCAAGCTGAAACTGACCAATTGGAA	MaxbHLH-Fos joint primer
9	gcta**CCATGG**GCAGCAGCGCGGATAAAGAA	eArntbHLH-Fos assembly
10	ACGACGTTCAATTTCGCTATGATTTTCACGCGCCAGACGTTCTTTATCCGCGCT	eArntbHLH-Fos assembly
11	GAAATTGAACGTCGTCGTCGTAATAAAATGACGGCGTATATTACCGAACTGAGCGATATG	eArntbHLH-Fos assembly
12	GGTCAGTTTATCCGGTTTACGCGCCAGCGCGCTGCAGGTCGGCACCATATCGCTCAGTTC	eArntbHLH-Fos assembly
13	CCGGATAAACTGACCATTCTGCGTATGGCGGTGAGCCATATGAAAAGCCTGCGTGGCACC	eArntbHLH-Fos assembly
14	CGGTTTCCGCCTGCAGGGTATTGCCGGTGCCACGCAGGCT	eArntbHLH-Fos assembly
15	TGCAGGCGGAAACCGATCAGCTGGAAGATGAAAAAAGCGCGCTG	eArntbHLH-Fos assembly
16	TTCTTTTTCTTTCAGCAGATTCGCAATTTCGGTCTGCAGCGCGCTTTT	eArntbHLH-Fos assembly
17	CTGAAAGAAAAAGAAAAACTGGAATTTATTCTGGCGGCGC	eArntbHLH-Fos assembly
18	GGTG**CTCGAG**ACGATGCGCCGCCAGAATAA	eArntbHLH-Fos assembly and eMaxbHLH-Fos primer
19	TT**CTCGAG**CATATACTGAATATATTCGGTCGC	eMaxbHLH primer
20	GCTA**CCATGG**GCGCGGATAAACGTGCGCAT	eMax construct forward primer
21	TTCGGTCGCTTTATCCAGAATCTGCGCACGGCTCGCTTTTTCGCCCTGCAGGCTCGG	eMaxbHLH-Fos joint primer
22	CTGGATAAAGCGACCGAATATATTCAGTATATGCAGGCGGAAACCGATCAGCTG	eMaxbHLH-Fos joint primer
23	GAATATATCCAGTATATGCAGGCGGAAACCGATCAGGCGGAAGATG	FosLA zipper assembly
24	CAGCGCATTCGCAATTTCGGTCTGCGCCGCGCTTTTTTCATCTTCCGCCTGATCGG	FosLA zipper assembly
25	GAAATTGCGAATGCGCTGAAAGAAAAAGAAAAAGCGGAATTTATTCTGGCGGCG	FosLA zipper assembly
26	ggtg**CTCGAG**ACGATGCGCCGCCAGAATAAATTCC	FosLA zipper assembly
27	GAATATATCCAGTATATGCAGGCGGAAATTGAACAGCTGGAAGAACG	FosW zipper assembly
28	GATCTTCAATTTCTTTGCGCAGCGCATAATTACGTTCTTCCAGCTGTTC	FosW zipper assembly
29	GCAAAGAAATTGAAGATCTCCAGAAACAGCTGGAAAAACTGGGAGCGCCG	FosW zipper assembly
30	ggtc**CTCGAG**CGGCGCTCCCAGTTTTTC	FosW zipper assembly

*Oligonucleotide sequences are shown in 5' to 3' direction. Restriction sites used for cloning are in bold.

Gene construction. DNA oligonucleotides were purchased from Operon Biotechnologies (Huntsville, AL) or Integrated DNA Technologies (Coralville, IA). The gene encoding ArntbHLH (residues 65-130) was constructed using the sequence from human Arnt isoform 3 (NCBI NP_848514) as previously described.[1] The sequence encoding ArntbHLH-Fos, a hybrid of human ArntbHLH (residues 82-149) fused to human c-Fos LZ (residues 166-202[2]), was constructed in a two-step manner: the TFos fragment containing the sequence encoding amino acids 143-149 of human Arnt followed by amino acids 166-202 of human c-Fos was assembled by self-priming PCR[3] using oligonucleotides 1-4 (all oligonucleotides used in this study are listed in Table S1), and the ArntbHLH sequence encoding amino acids 82-149 of the bHLH domain of human Arnt isoform 1 was amplified from the $Arnt_{82-464}$ cDNA fragment[4] using oligonucleotides 5 and 6 as the 5' and 3' primers, respectively. The final ArntbHLH-Fos fragment was then PCR-amplified with TFos and ArntbHLH described above as templates, using oligonucleotides 5 and 4 as the 5' and 3' primers, respectively.

The MaxbHLH sequence encoding residues 22-74 of murine Max protein[5] was cloned from pGAD424/MaxbHLH[6] using EcoRI and BamHI restriction sites. The sequence encoding MaxbHLH-Fos, a hybrid of murine MaxbHLH (residues 22-74) fused to human c-Fos LZ (residues 166-202[2]), was constructed by PCR similar to that described above for ArntbHLH-Fos. The XFos fragment containing the sequence encoding amino acids 66-74 of murine Max followed by amino acids 166-202 of human c-Fos was assembled by self-priming PCR[3] using oligonucleotides 2-4 and 8 (Table S1). The final MaxbHLH-Fos fragment was then PCR-amplified with XFos and MaxbHLH described above as templates, using oligonucleotides 7 and 4 as the 5' and 3' primers, respectively.

The DNA sequences described above were inserted into vector pGADT7-2 (equivalent to pGAD424-MCS II in ref. [7]), a GAL4 AD plasmid constructed by replacing the MCS of pGAD424 (Matchmaker™ One-Hybrid System, Clontech) with that of pGADT7 (Matchmaker™ Two-hybrid System, Clontech). Recombinant plasmids were then transformed into *E. coli* strain DH5α, harvested, and sequences confirmed by DNA sequencing (The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON).

Gene assembly for protein expression. The DNA fragments encoding ArntbHLH-Fos, MaxbHLH, MaxbHLH-Fos, MaxbHLH-FosLA, and MaxbHLH-FosW in *E.coli*-preferred codons were assembled. ArntbHLH-Fos was assembled by self-priming PCR[3] using oligonucleotides 11-18. MaxbHLH was PCR-amplified from pET28a(+)/MaxbHLHZ[6] using primers 19 and 20. MaxbHLH-Fos was generated in two steps. In the first step, MaxbHLH was amplified from pET28a(+)/MaxbHLHZ using primers 20 and 21 while the Fos zipper was amplified from pET28a(+)/ArntbHLH-Fos using primers 22 and 18. The final MaxbHLH-Fos fragment was then PCR-amplified with Fos and MaxbHLH described above as templates using oligonucleotides 20 and 18 as the 5' and 3' primers,

respectively. The MaxbHLH-FosLA and MaxbHLH-FosW constructs were assembled in a similar manner, but for these constructs, the zipper fragments were assembled by self-priming PCR[3] using oligonucleotides 23-26 for FosLA and 27-30 for FosW. All constructs described above were cloned into restriction sites NcoI and XhoI in vector pET28a(+) (Novagen, Mississauga, ON). Recombinant plasmids were then transformed into *E. coli* strain DH5α, harvested, and sequences confirmed by DNA sequencing (The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON).

Arnt Isoforms 1 and 3. Both Arnt isoforms 1 and 3 are used in the lab. In this study, however, Arnt isoform 1 was utilized as some results presented here are part of continuing studies with isoform 1 (unpublished results, planned submission Spring 2012) and previous results.[4]

Moreover, it has been reported that Arnt isoforms 1 and 3 behave in a similar manner in TCDD-induced signal transduction.[8] As for DNA-binding function, the Whitelaw lab found that phosphorylation of Ser77 had an inhibitory effect on DNA-binding to E-box by isoform 1 homodimers but not isoform 3 homodimers.[9] In our case, Ser77 is not included in the sequence under study. Therefore, although we used Arnt isoforms 1 and 3 for comparison, they should behave similarly regarding DNA-binding function. Furthermore, our conclusion was based on both Arnt and Max constructs, and the difference between the Arnt constructs does not affect our conclusion.

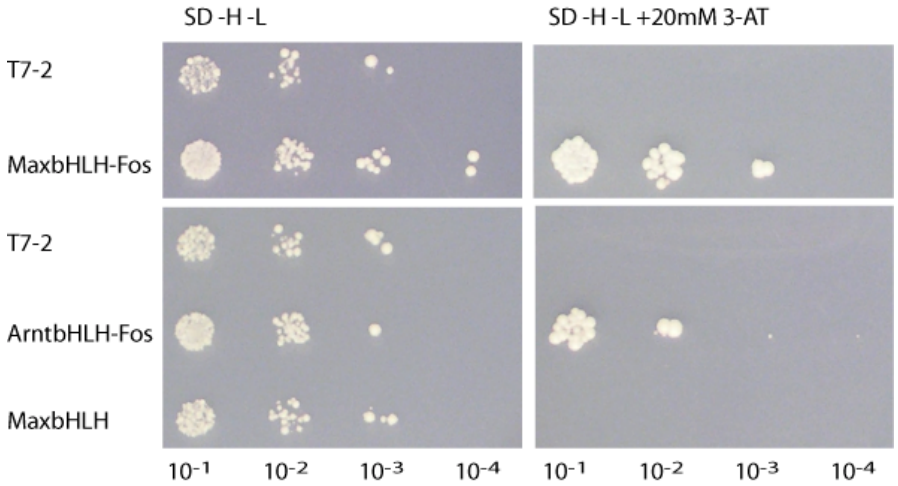

Figure S1. The *HIS3* assay. Vectors pGADT7-2 expressing different proteins as C-terminal fusions to GAL4 AD were transformed into yeast strain YM4271[pHISi-1/E-box]. The resulting yeast colonies expressing these GAL4 AD fusions are listed along the left, with control expressing GAL4 AD only in the first and third rows. Colonies were spotted (10 μL) as ten-fold serial dilutions onto SD/-His/-Leu medium to select for the presence of plasmid, followed by spotting on SD/-His/-Leu medium with 20 mM 3-AT to select for DNA-binding protein leading to colony growth.

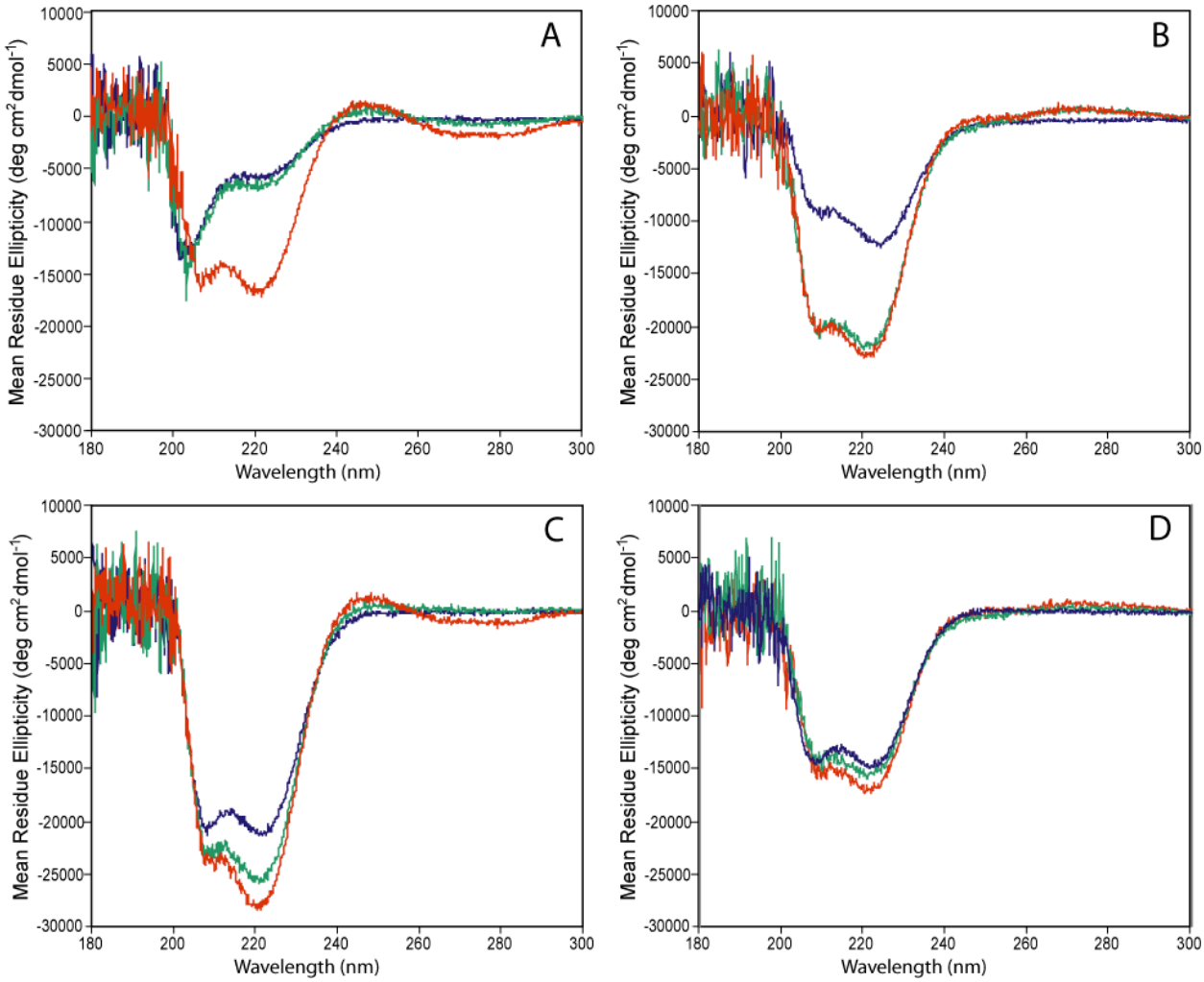

Figure S2. Circular dichroism. Spectra of **(A)** MaxbHLH-FosLA, **(B)** MaxbHLH-Fos, **(C)** MaxbHLH-FosW, and **(D)** MaxbHLHZ in the absence of DNA (blue), with nonspecific DNA (green), or Max E-box DNA (red). DNA sequences were the same as those used for fluorescence anisotropy (Figure 3) but without 6-FAM. Samples contained 2 μM protein monomer and 2 μM DNA where appropriate. Each spectrum was averaged twice, and curves were not subjected to smoothing. The buffer control was subtracted from each spectrum. Mean residue ellipticities are presented, which account for differences in lengths of proteins.

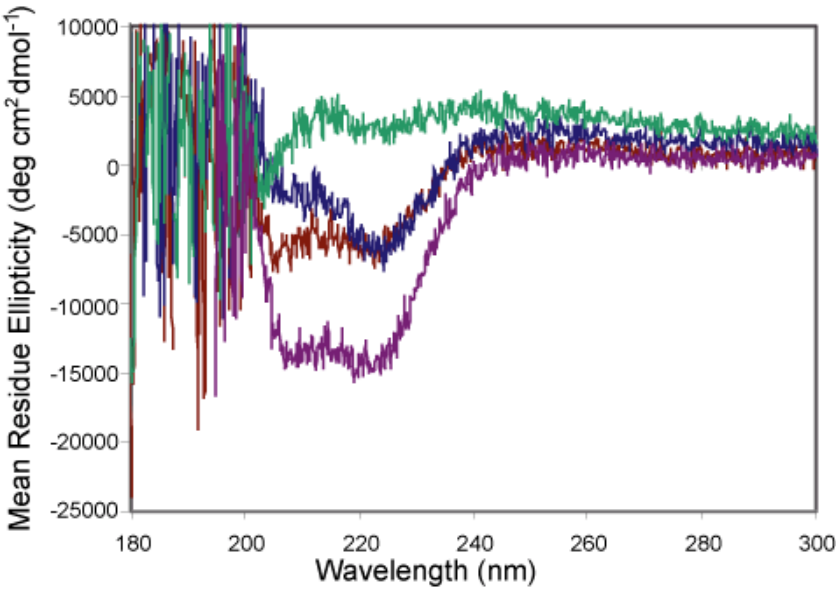

Figure S3. Circular dichroism spectra (from top to bottom) of MaxbHLH-FosLA (green), MaxbHLH-Fos (dark blue), MaxbHLHZ (red), and MaxbHLH-FosW (purple). Samples contained 0.5 μM protein monomer. Each spectrum was averaged twice, and curves were not subjected to smoothing. The buffer control was subtracted from each spectrum. Mean residue ellipticities are presented, which account for differences in lengths of proteins.

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