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Short Communication

Organization of the human FK506-binding immunophilin FKBP52 protein gene (*FKBP4*)☆

Jonathan G. Scammell,[a,b,*] Tina R. Hubler,[a] Wesley B. Denny,[a] and Donna L. Valentine[a]

[a] *Department of Pharmacology, University of South Alabama College of Medicine, Mobile, AL 36688, USA*
[b] *Department of Comparative Medicine, University of South Alabama College of Medicine, Mobile, AL 36688, USA*

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Abstract

FKBP52 is a widely expressed FK506-binding immunophilin that possesses peptidylprolyl isomerase activity and a tetratricopeptide repeat involved in protein–protein interaction. FKBP52 plays an important role in steroid receptor function and is implicated in other diverse processes, including regulation of transcription, cation channel activity, and gene transfer efficiency. Reported here is the genomic organization of the human FKBP52 gene (*FKBP4*), which shares all but one of the same exon–intron boundaries as the structurally related immunophilin FKBP51 gene (*FKBP5*). Approximately 3.5 kb of 5′-flanking DNA of *FKBP4* was subcloned into a luciferase reporter vector and was found to exhibit robust activity in T-47D, MCF7, and COS-7 cells. Promoter constructs with only 143 bp of upstream sequence maintained high activity. This region contains a CAAT motif sequence and consensus binding sites for Sp1, heat-shock factor, and MYC-MAX, which are conserved in the rabbit *FKBP4* promoter and, when deleted, dramatically reduced promoter activity in T-47D cells.

Keywords: Immunophilin; FK506; Genes, reporter; Luciferase; Promoter; Exons; Introns; Transcription factors

FKBP52 (also referred to as hsp56, p59, or FKBP59) is a high-molecular-weight, FK506-binding immunophilin that was first identified as a component of steroid hormone receptors [1]. cDNAs encoding rabbit and human FKBP52 were isolated in 1992 [2,3] and that of the mouse protein was cloned a year later [4]. Analysis of transcript and protein levels has demonstrated that FKBP52 is widely expressed in human and rabbit tissues [3,5,6]. Structurally, FKBP52 resembles the other high-molecular-weight immunophilins with an N-terminal peptidylprolyl *cis–trans* isomerase (PPIase) domain and a C-terminal tetratricopeptide repeat (TPR) domain, which mediate protein–protein interactions involved in FKBP52-regulated cellular functions [7]. For example, FKBP52 is incorporated into steroid receptor complexes via TPR domain binding to hsp90 [8] and facilitates translocation of the activated receptor to the nucleus via PPIase domain binding to dynein [9]. Constitutively low expression of FKBP52 is thought to contribute to glucocorticoid resistance in New World primates [10,11]. In addition to its role in steroid receptor complexes, FKBP52 serves as a transcriptional regulator by repressing the activity of interferon regulatory factor-4 in immune cells [12] and heat-shock factor 1 in HeLa cells [13]. FKBP52 has been implicated in the cardiotrophic effect of cardiotrophin-1 [14], in the neuroprotective effects of FK506 [15], and in regulating the efficiency of adeno-associated virus type 2-mediated transgene expression [16]. The *Drosophila* homolog of FKBP52, dFKBP59, regulates cation channel activity in photoreceptor cells [17]. Thus, FKBP52 may have regulatory roles in diverse physiological and biochemical processes.

Despite intense investigation aimed at understanding the functions of FKBP52 there has been only modest progress in elucidating the molecular biology of FKBP52. In 1998, the human FKBP52 protein gene (*FKBP4*) was mapped to the short arm of chromosome 12 [18]. More recently, Davis and colleagues compared the exon–intron organization of

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* Corresponding author. Fax: +1-251-460-6798.
E-mail address: jscammel@jaguar1.usouthal.edu (J.G. Scammell).

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Table 1
Splice junction sequences of the *FKBP4* gene

Exon		Intron–exon junction sequence		Intron	
Number	Size (bp)	3′-splice acceptor site	5′-splice donor site	Letter	Size (kb)
1	258	—	CTGAAG^{258}**gt**gagg	A	1.9
2	145	tccc**ag**^{259}GTCATC	GAAAAG^{403}**gt**aggc	B	0.4
3	143	ctgc**ag**^{404}GGGAGG	TTTGAG^{546}**gt**gagt	C	0.8
4	121	ccac**ag**^{547}GTGGAG	TGGAGG^{667}**gt**gaga	D	0.3
5	157	tgcc**ag**^{668}TTGCAG	GCCCAG^{824}**gt**gagg	E	0.6
6	91	gtcc**ag**^{825}CTATGC	GAAAAG^{915}**gt**aagt	F	0.1
7	84	tggc**ag**^{916}GCCAAG	TTCAAG^{999}**gt**gagc	G	0.3
8	186	ttcc**ag**^{1000}GAAGGT	AACAAG^{1185}**gt**gagg	H	0.5
9	240	cttt**ag**^{1186}GCCCTA	AACAAG^{1425}**gt**gagg	I	1.8
10	>800	ttgc**ag**^{1426}GCCAAG	—		

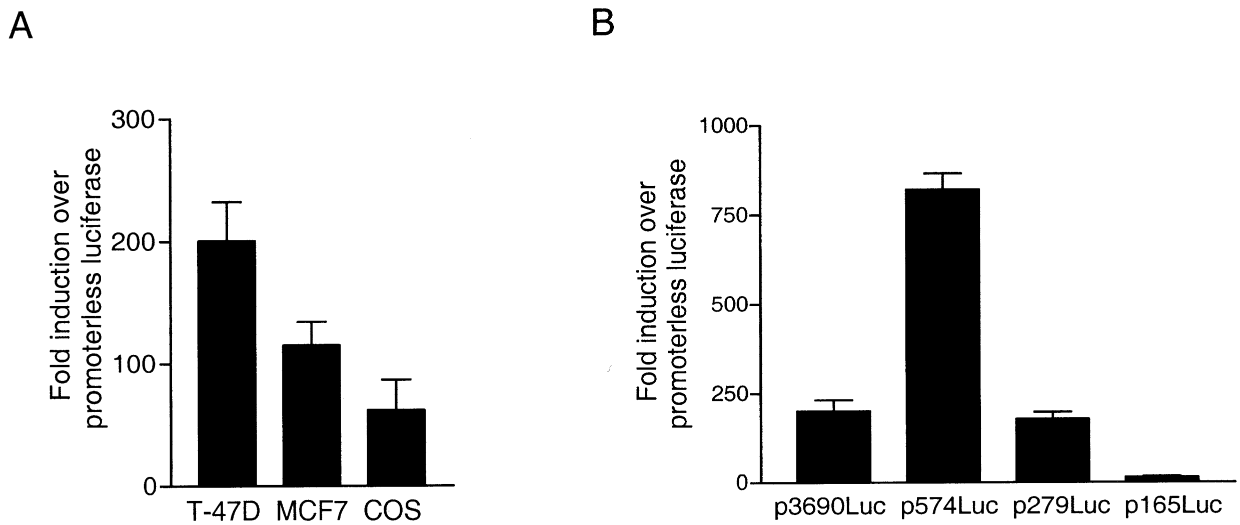
Note. Exon–intron boundaries were determined by comparison of sequences of genomic DNA and cDNA (GenBank Accession Nos. AC005841 and NM_002014, respectively). Upper- and lowercase letters represent the exons and introns, respectively. The nucleotide positions of the splice sites are displayed as superscripts, and they correspond to the nucleotide position number in the human FKBP52 (*FKBP4*) cDNA (NM_002014).

the PPIase domains of the mouse FKBP65 gene (*FKBP10*) and other FKBP gene family members including *FKBP4* [19]. They demonstrated that the organization within the PPIase domains of *FKBP4* and the structurally related FKBP51 gene (*FKBP5*) is identical and distinct from most other FKBPs. However, the organization of the regions of *FKBP4* coding for other domains of FKBP52 was unknown as were the details of the *FKBP4* promoter. We report here the complete organization of *FKBP4* and isolation and expression of the *FKBP4* promoter in a cell-type-specific manner.

Exon–intron boundary positions were deduced from sequence comparisons between genomic DNA (AC005841, human chromosome 12p13.3) and the human FKBP52 cDNA sequence (NM_002014). We found that the *FKBP4* consists of 10 exons and 9 introns spanning approximately 9 kb of genomic DNA (Table 1). The organization of *FKBP4* is identical to that of the structurally related immunophilin *FKBP5* with the exception that *FKBP5* has an additional intron in the 5′-noncoding sequence [11]. Introns in human *FKBP4* are significantly shorter (0.1 to 1.9 kb) than those of *FKBP5* (0.9 to 46 kb). We noted slight deviations from the previous report [19] in the positions of the first two exon–intron boundaries of *FKBP4* resulting in V^{46} falling in exon 2 and G^{84} falling in exon 3. Each donor and acceptor site conformed to the consensus GT-AG sequence [20]. *FKBP4* and *FKBP5* share some similarity in intron positions with the gene for the FKBP family member FKBP36 [19], but there is little similarity with genes of other FKBP family members or with the gene for the TPR-containing immunophilin cyclophilin 40, which like FKBP51 and FKBP52 is associated with steroid hormone receptors [7,21].

A fragment of 5′-flanking DNA of *FKBP4* was amplified by PCR using primers 5′-TATACGCGTAGGACTCACACG-3′ and 5′-TACAGATCTTTATCCGGGAGC-3′, corresponding to sequences approximately 3.5 kb upstream of exon 1 and positions 135–124 of the human FKBP52 cDNA within exon 1 (NM_002014), flanked by *Mlu*I and *Bgl*II sites (underlined sequences), respectively. The amplified fragment (−3554 to +136) was subcloned into the pGL3-Basic vector (Promega Corp., Madison, WI, USA). Plasmid construction was confirmed by restriction enzyme analysis and DNA sequencing of the 5′ and 3′ ends. The activity of the *FKBP4* promoter construct (p3690Luc) was tested in human T-47D and MCF7 breast cancer and African green monkey COS-7 kidney cells. FKBP52 is constitutively expressed in most cell types but is expressed at high levels in many breast cancer cell lines including T-47D and MCF7 cells [22]. The activity of p3690Luc was 200- and 115-fold higher in T-47D and MCF7 cells, respectively (Fig. 1A), than that of the promoterless pGL3-Basic vector, which was transfected into parallel sets of cells to account for differences in transfection efficiency. The activity of p3690Luc in COS-7 cells was also robust (71-fold higher than the promoterless vector).

Next, we investigated the effects of deletions of 5′ DNA of the *FKBP4* promoter on activity in T-47D cells. 5′ deletions were generated using p3690Luc as the template and ligation into pGL3-Basic as follows: p574Luc (−438 to +136), by restriction digestion with *Sma*I and *Bgl*II; p279Luc (−143 to +136) and p165Luc (−29 to +136), by PCR amplification with 5′-AGCACTGAGCCCGGGCCGGCTCAGTCC-3′ (*Sma*I site underlined) and 5′-CCAATCGCTCCCCGAGCTCCTCCTGACCCACCTACC-3′ (*Sac*I site underlined), respectively, and the reverse primer described above. The nucleotide sequence of the promoter insert of p574Luc was determined across both strands and shows marked homology with the first 150 bp of the rabbit *FKBP4* proximal promoter (Fig. 1C). As attempts to determine the transcription initiation site of *FKBP4* were unsuccessful, likely due to the high GC content of this region, the first base of the 5′ untranslated region of the FKBP52 mRNA (NM_002014) was designated +1. There was no clear TATA-like motif sequence, but a CAAT box motif,



C

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                                                                              ▼
human                                                                         AGGG GGCGCCGCCA CTGCCATCCC GCAGCCTGCG CTCCCCACAC CCCGCCGCAG  -385
                                                  CDP CR→                                                                     MZF1→
human        GCGCAGCGGA CTGGTGGAGT GATGTCGGGA GATCGATCGA GAAGAATCTG GAAGAATCCG GTTAGACAGG GAAGCGGGCG GGGATGGGAG GGAGGGAGGA  -285
                                                                             MZF1→                                   CP2→
human        AGGGCCAAGG GAGGCCGCCG GGGAGATCCC GAGTCCGAGG GTCCCAGGAG AAGGGGGAGG GGGCAGGCAG CCAGAAGGAG GCAAGAACCA AAAAAAAAAA  -185
                                                         ▼                                 HSF→        ←Sp1
human        AAGAAAAAAA AATTCCGAAG CGCCCGCAGC ACTGAGCCCG AACCGGCTCA GTC*CCGCCG TCTCTAGAAA GTTCTGCACC CC*GCCCCCT CTGTCT****  -90
rabbit                             TAAAGCACTA -AAC----A- ---------G -C-G------ A--------- -----C-G-- --C------- -C----CCCG
                                   ←Sp1       MYC→  Sp1→      CAAT box                 ▼                                 +1
human        ***GCGCTCC CGCTCCTCCC AGCCCCTCCC TCGCGGCGGG GCGGGACCAA TCGCTCCCCG AGCCGCTCCT GACCCACCTA CCCCAGCTC* **TCGCGCCG  +4
rabbit       CTC----G-- A--------- ---------- ---------- -T-------- ----G---TC ------*-G- CCA--T--GC -G-GG----C GAG-------

human        CGTGCAGAGG TGCTCAAGCC TCCTCGCGGT CCGCAGTCAG TGCCGCCGCG CCCGGCCTC* CCGCACGCCC CGCAGGTAGC GCCCCCGCCC GCGGCCCAGA  +103
rabbit       GC------*- A--G-G--*- -G-GGC-A-- -----CG-CC G--A-----C AGT-C-G--G ---------T -A-*--GGCA -GT--G--GT T---GAGG-T

human        GTGCGCTCGC GCCGGCACCA GCTCCCGGAT AAA                                                                           +136
rabbit       T--*------ --T-CGCT-G -TC-A----C TCA
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Fig. 1. (A) Cell-specific expression of an FKBP4 promoter–luciferase fusion gene. T-47D, MCF7, and COS-7 (COS) cells were transfected with either p3690Luc or the promoterless pGL3-Basic vector (0.5 μg/well) using 3 μg Superfect (Qiagen) for 2 h. The cells were collected after 24 h for determination of luciferase activity, which was expressed as fold induction over the activity of pGL3-Basic. Each bar represents the mean ± SEM of three separate experiments. (B) Effect of progressive 5′ deletions on the activity of the FKBP4 promoter in T-47D cells. Experiments were performed as described above. Each bar represents the mean ± SEM of three separate experiments. (C) Comparison of the promoters of the human and rabbit FKBP4 (GenBank Accession Nos. AY167569 and AF383153). Nucleotides are numbered from the transcription initiation site of the human FKBP4 (+1). Bases that are conserved between human and rabbit sequences are indicated with dashed lines. Gaps are indicated by asterisks. DNA consensus binding sites for transcription factors were identified using the TRANSFAC TFSEARCH program and are either underlined or indicated in bold (MYC). Inverted arrows indicate the 5′ ends of each of the deletion constructs.

conserved in the rabbit *FKBP4* promoter, was identified at positions −42 to −49.

The activities of each of these promoter deletion constructs were compared with that of p3690Luc. Deletion of 3 kb of upstream DNA from p3690Luc resulted in a 4-fold increase in promoter activity, p574Luc exhibiting an activity in T-47D cells which was more than 800-fold higher than achieved with the promoterless vector pGL3-Basic (Fig. 1B). Further deletion of 5′ DNA to yield a construct containing 143 bp of upstream sequence (p279Luc) reduced activity by 4.5-fold, although the activity of this construct was still 180-fold higher than that of pGL3-Basic. On the other hand, when the *FKBP4* promoter was deleted to only 29 bp of upstream sequence (p165Luc) activity was reduced by greater than 90% compared to p279Luc. A similar pattern of activity was observed when the different constructs were transfected into MCF-7 cells (data not shown). These results suggest that the first 143 bp of the *FKBP4* promoter are sufficient to support robust expression in breast cancer cells and that 3.5 kb of upstream DNA contain both stimulatory and inhibitory *cis*-acting elements.

Possible transcription factor binding sites within the first 438 bp of the *FKBP4* promoter were identified using the TRANSFAC TFSEARCH program version 1.3 [23] (Fig. 1C). The first 140 bp of the proximal promoter contain several GC boxes (consensus sequence GGGGCGGGG), which are important for the expression of many ubiquitously expressed genes and bind Sp1 [24]. This region also contains a consensus binding site for heat-shock factor (HSF; core sequence GAAnnTTC [25]), which may contribute to constitutive and/or stimulated expression of *FKBP4*. For example, elevated expression of HSF2 may contribute to high FKBP52 levels in testes [5,6,26]. In addition, activation of this region by HSF1 may mediate the induction of FKBP52 mRNA by heat shock

[27]. We also identified a MYC-MAX-like binding site (consensus sequence CACGTG [28]), which may mediate the induction of FKBP52 mRNA in cells constitutively expressing MYC [29]. Each of these sequences is conserved in the rabbit *FKBP4* promoter. Further upstream, potential binding sites for CP2, MZF1, and CDP CR1 were identified, although they do not likely play a functional role in constitutive expression of *FKBP4* in breast cancer cells, as a construct in which these elements were eliminated (p279Luc) had activity similar to that of the longest construct we tested (p3690Luc). Sp1 sites are also present in the proximal promoter of *FKBP5* [30], whereas in the cyclophilin 40 gene GA binding protein binding sites appear to play a critical role in constitutive expression [30]. Isolation of the *FKBP4* promoter presented here will allow for detailed analysis of how this immunophilin gene is regulated in normal and cancer cells.

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