PERGAMON

Journal of Steroid Biochemistry and Molecular Biology 69 (1999) 323–328

The Journal of Steroid Biochemistry & Molecular Biology

Human Ad4BP/SF-1 and its related nuclear receptor ☆

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Abstract

Ad4BP (or SF-1) is an essential transcriptional factor for steroidogenesis as well as for the development of the reproductive axis. We elucidated the structure of the human Ad4BP gene. The spliced variants of Ad4BP gene, ELP1 and ELP2 in mice, are unlikely to be present in humans since the analysis of the human gene revealed an in frame stop codon, 36-bp before the first ATG of Ad4BP. The promoter sequence of human Ad4BP, upstream of non-coding exon 1 was highly conserved, and E-box was also found to be essential for the transcription of human Ad4BP gene. During the process of the human Ad4BP gene cloning, we happened to obtain an Ad4BP-related gene, FTZ-F1β which also belongs to the nuclear receptor family. We revealed cDNA structures of rat FTZ-F1β, and found that rat has at least two types of FTZ-F1β isoforms, which differ only by 21 amino acids length in the A/B domain. The tissue distributions of FTZ-F1β in rat examined by RT-PCR, was found to be abundant in liver, pancreas, and gastrointestinal tracts. These results suggest that the physiological significance of FTZ-F1β is different from that of Ad4BP.

1. Introduction

Ad4BP (SF-1) was originally identified as a steroidogenic tissue-specific transcription factor which regulates the expression of steroidogenic P450 hydroxylases in adrenocortical cells [1–5]. This factor is a mammalian homologue of Drosophila's fushi tarazu factor (Ftz-F1) [6] and structurally belongs to a member of the nuclear receptor superfamily that includes receptors for the steroid, thyroid, and retinoid hormones [3,7]. The Ad4BP binds to the sequences PyCAAGGPyC or PuPuAGGTCA [1,8] of various steroidogenic P450 genes [1,5,8] and steroidogenic acute regulatory protein (StAR) gene [9], thus leading to the hypothesis that Ad4BP might be a shared transcriptional factor that determines the expression of steroidogenic genes. Indeed, the actual involvement of Ad4BP in both cAMP-responsive and tissue-specific transcription of various steroidogenic genes has been reported [5]. The disruption of the mouse Ftz-F1 has been shown to cause a complete lack of adrenal glands and gonads [10] as well as a selective loss of gonadotropin-specific markers such as LH-β, FSH-β, and GnRH receptor in the pituitary [11,12]. Ad4BP/SF-1 is thus thought to be essential for the development of a functioning hypothalamus-pituitary-adrenal and gonadal axis.

On the other hand, a nuclear receptor, mouse LRH-1 structurally corresponds to a counterpart of Ad4BP [13]. A transcription factor called FTF that controls the expression of rat α1-fetoprotein gene turned out to be the ortholog of mLRH-1 [13]. During the process of Ad4BP gene cloning, we happened to obtain a unique clone which hybridizes with Ad4BPcDNA but is clearly different from the human Ad4BP gene. The finding prompted us to investigate the structural comparison between Ad4BP/SF-1 and LRH-1/FTF.

2. Structure and its regulation of human Ad4BP gene

We have cloned human Ad4BP gene and clarified its structure [14]. This gene is at least 28 kb long and is

☆ Proceedings of the Xth International Congress on Hormonal Steroids, Quebec City, Quebec, Canada, 17–21 June 1998.

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0960-0760/99/$ - see front matter
PII: S0960-0760(99)00081-3

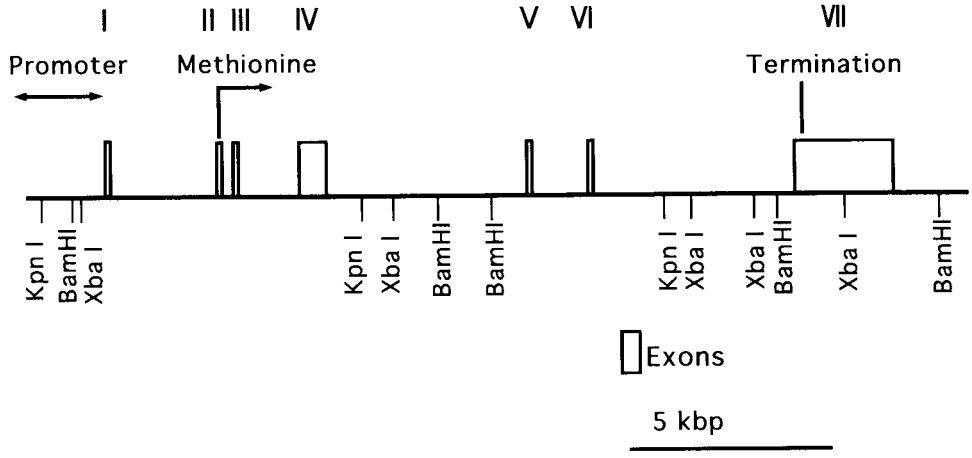


Fig. 1. Structure of human Ad4BP gene. The open boxes indicate the locations and sizes of exons. The Methionine in exon II indicates translation initiation site, whereas Termination in exon VIII indicates the translation termination site.

split into 7 exons including a non-coding exon 1 by 6 introns (Fig. 1). The coding sequence of human Ad4BP gene was highly conserved, since it was 88, 88, and 93% identical at the nucleotide level, and 93, 93 and 94% identical at the amino acid level, to those of rat, mouse and bovine, respectively. The deduced amino acid sequence of the human Ad4BP consists of 461 amino acid residues. Clarification of the primary structure of mouse, rat, bovine and human Ad4BP/SF-1 revealed two mammalian variants with 461 or 462 amino acids, which differ by the absence or presence of a single glutamine residue at the amino acid position 206. The human and bovine Ad4BP/SF-1 proteins belong to a group without the glutamine residue, while mouse and rat Ad4BP/SF-1 proteins belong to a group with the glutamine residue. Southern blot analysis after digestion of human genomic DNA with several restriction enzymes showed simple pattern of digestion, suggesting that human Ad4BP gene is a single copy gene. All exon-intron boundaries follows the 'GT-AG rule' for the splice donor and acceptor [14].

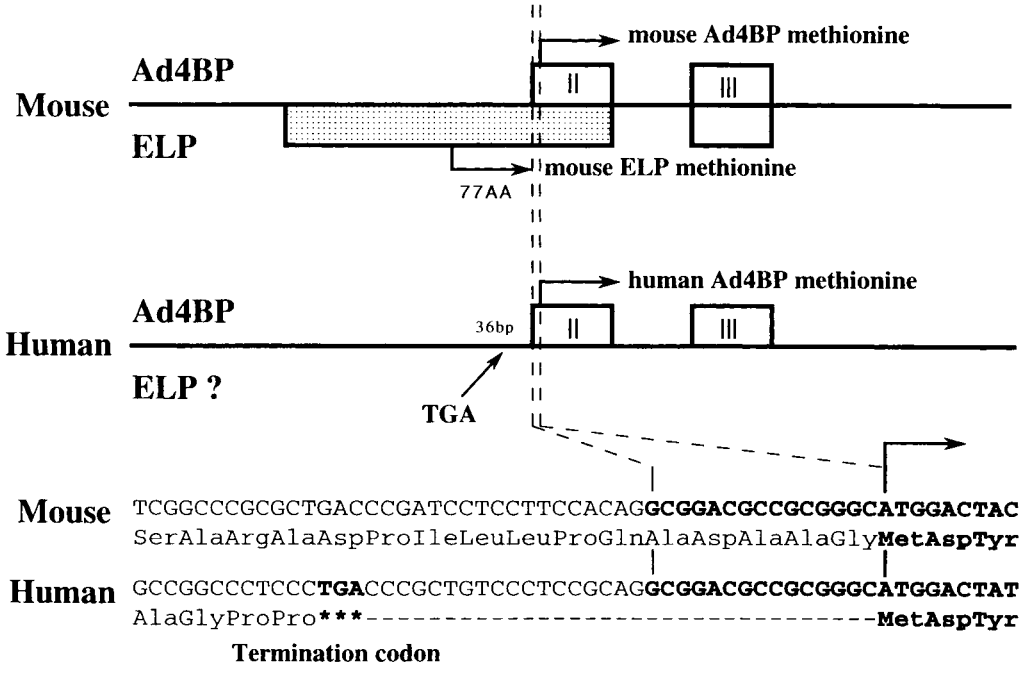


Fig. 2. Genetic difference of the nucleotide sequences around translation initiation site between human and mouse Ad4BP/SF-1 gene.

Table 1
Molecular basis of the patients with congenital adrenal hypoplasia (primary adrenal insufficiency) and hypogonadotropic hypogonadism[a]

Patient	Age/Sex	Race	Inheritance	DAX-1	Ad4BP
1	17/M	Japanese	X-linked	Trp 171 Stop	
2	18/M	Japanese	X-linked (sporadic)	1-bp deletion (codon 280)	
3	20/M	Japanese	X-linked	1-bp deletion (codon 49)	
4	28/F	Japanese	Autosomal recessive?		Normal
5	2/F	Thai	Autosomal recessive?		Normal
6	11/F	Thai	Autosomal recessive?		Normal

[a] Genomic DNA of the patients was extracted from leukocytes [25]. The coding region of the DAX-1 gene [20] or Ad4BP gene [14] was amplified from 1 μg genomic DNA to obtain segments for determination of the nucleotide sequence using polymerase chain reaction (PCR) [26]. The PCR was performed 30–35 amplification cycles using the Taq DNA polymerase (Cetus Corp., Emneryvillee, CA), the DNA Thermo Cycler (Perkin–Elmer Cetus) and appropriate sense/antisense primers. The PCR products were run on 1.2% agarose gel, purified by QIAEX II (QIAGEN, Germany), and directly sequenced by the dideoxynucleotide method [27] using the ALFexpress DNA Autosequencer (Pharmacia Biotech, Uppsala, Sweden).

Embryonal long-terminal repeat-binding protein (ELP), originally identified as a repressor of retroviral gene expression in embryonal carcinoma cells [15] is produced from the same gene encoding Ad4BP by alternative promoter usage and splicing [4,16,17]. However, the functional significance of ELP in mammalian spices is unknown. It has been reported that classical ELP, ELP1 or ELP2 in mouse was transcribed within the Ad4BP first intron and 77 more amino acid residues are translated preceding the Ad4BP initiation methionine [11]. In contrast, as shown in Fig. 2, a predicted structure of ELP in human was rather different from that of mouse: namely, the analysis of the human Ad4BP gene in our study revealed an in frame stop codon, TGA 36-bp before the first ATG of Ad4BP. Thus, at least, classical and the most subtype of ELP corresponding to ELP1 and ELP2 in mice is unlikely to be present in humans [14]. A similar kind of structural alteration of ELP transcripts by premature termination has also been demonstrated in rat Ad4BP gene [16]. Such great structural divergence of the ELP gene among mammalian species may suggest reduced physiological significance of ELP.

We mapped the major transcription start site of human Ad4BP gene in the human adrenal gland and the start site was a few bases up or down stream of that of mouse or rat [14]. The promoter sequence of human Ad4BP, upstream of non-coding exon 1, was also highly homologous to those of rat and mouse; and most of the consensus sequences for binding sites of transcriptional factor such as GATA-1, E-box, AP-2 and CCAAT box were preserved, suggesting a shared, and essential regulatory mechanism in the expression of this gene beyond species [14]. On the regulation of the Ad4BP gene expression, a cis-acting element, E box located at around −80 in the promoter region and its binding protein are reported to be essential for the expression of rat and mouse Ad4BP gene [17,18]. The deletion analysis of 5'-flanking region of the human Ad4BP gene revealed that E box is also essential to the basal expression of human Ad4BP gene (unpublished observation).

3. The human Ad4BP gene and its related disease

Another important question to address is whether Ad4BP is related to disease or not. SF-1 knockout mouse completely lacks adrenals and gonads as well as a selective loss of gonadotropin expression. Interestingly, the phenotype of the SF-1 knockout mouse [10] is, if not completely the same, very similar to that observed in a subset of human patients who show congenital adrenal hypoplasia complicated with hypogonadotropic hypogonadism [19]. Congenital adrenal hypoplasia is a rare disorder manifesting as primary adrenal insufficiency usually early in infancy, with low serum concentrations of glucocorticoids, mineralocorticoids, and adrenal androgens. Most cases having congenital adrenal hypoplasia and hypogonadotropic hypogonadism are male thus showing X-linked recessive inheritance [19]. This type has been proven to be caused by fatal mutations of the DAX-1 gene on the X-chromosome [20–23]. Thus, DAX-1 is thought to be essential for the development of a functioning hypothalamus-pituitary-gonadal axis. However, exceptional cases, with congenital adrenal hypoplasia and hypogonadotropic hypogonadism, such as female patients and patients with autosomal recessive inheritance, have also been reported, and the molecular basis of such patients remains unclear [22]. Since human Ad4BP has been mapped on autosomal chromosome 9 [24], the human Ad4BP/SF-1 gene could be a candidate gene underlying such exceptional cases.

We have analyzed the molecular basis of six cases with congenital adrenal hypoplasia (primary adrenal

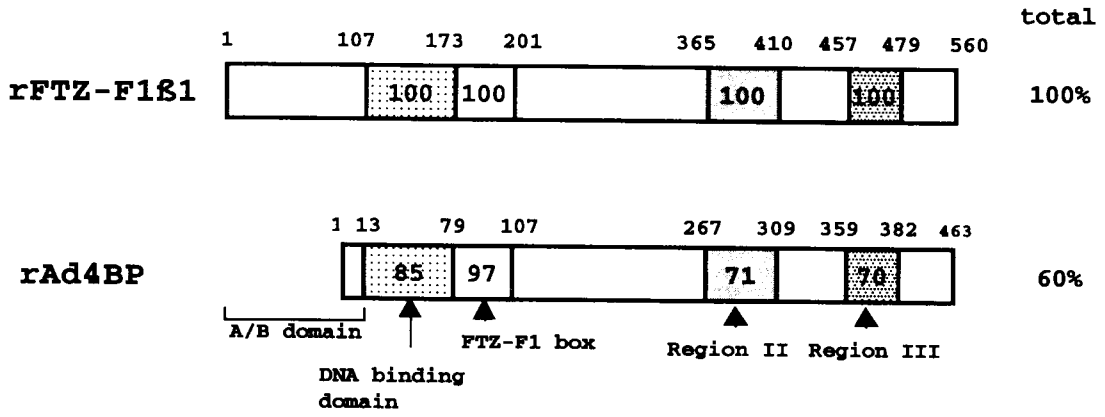


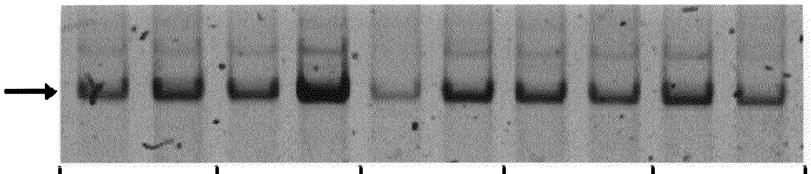
Fig. 3. Structural comparison between the rat Ad4BP/SF-1 and rat FTZ-F1β1 cDNA. Rat Ad4BP/SF-1 structure was cited from [16]. The percentage of amino acid homology of each structural domain between these two cDNAs was indicated when that of rat FTZ-F1β1 was considered to be 100%.

insufficiency) complicated with hypogonadotropic hypogonadism (Table 1). The three male cases (cases 1–3) had mutations in DAX-1 genes; all are nonsense mutations by themselves or as a result of alterations of the reading frame, thereby leading to the production of a truncated inactive protein [22,23]. However, the other three cases (cases 4–6) are exceptionally, female patients, and thereby difficult to explain by DAX-1 abnormalities; so we analyzed Ad4BP genes of these patients. However, no mutations were found in the coding regions and E-box of Ad4BP genes of these patients. These results suggest that other than DAX-1 or Ad4BP/SF-1, there may be another important autosomal gene responsible for the development of pituitary adrenal and gonadal axis.

4. Ad4BP-related gene

During the process of Ad4BP gene cloning, we happened to obtain a unique clone which hybridizes with Ad4BP cDNA but is clearly different from the human Ad4BP gene. Retrospectively, the clone turned out to be a partial gene of the human counterpart of the mouse liver receptor homologue-1 (mLRH) [13].

Mouse LRH-1 structurally corresponds to a



	N C	N C	N C	N C	N C
Age Sex	75 F	50M	60M	40M	59M
Disease	HCC	HCC	CCC	CCC	HCC
AFP	8013	8.0	12.0	160.3	7.7
Expression Ratio(C/N)	1.83	4.40	7.31	0.62	0.60

Fig. 4. Expression of FTZ-F1β mRNA in hepatocellular carcinoma (HCC) and cholangiocellular carcinoma (CCC) examined by RT-PCR. Total RNA was extracted from the tissue obtained at surgery and RT-PCR was performed for the rough evaluation of FTZ-F1β mRNA. A 310 bp of DNA fragment was amplified by PCR using sense/antisense primers, 5′-GTAGTCTGATGTGTCCTTCC-3′/5′-CTTGCATGTTTTCCGGCTTCT-3′. The PCR was performed with 22 amplification cycles (denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 3 min) using the Taq DNA polymerase and the DNA Thermo Cycler. The PCR products were run on 5% polyacrylamide gel and stained with Vitragreen (Amersham Co.), so as to detect the small amount of PCR products by low cycle numbers. The serum concentration of α-fetoprotein (ng/ml) in each patient was indicated.

counterpart of Ad4BP/SF-1. Mouse LRH-1 cDNA was originally cloned from mouse liver by Green et al. (GenBank No. M81385) one or two years before the clarification of SF-1 or Ad4BP cDNA structure [2,3]. We elucidated the primary structure of two types of rat LRH-1/FTF, namely, rFTZ-F1β1 and rFTZ-F1β2 (unpublished observations). We named our cDNAs rFTZ-F1β1 and β2, rather than rFTF1 and rFTF2, based on the classification of nuclear receptors advocated in 1995 [7]. In this classification, Ad4BP/SF-1 and mLRH-1 were classified as α and β forms of the FTZ-F1 family, respectively. The shorter form, rFTZ-F1β2, showed a predominant expression over the longer form, β1, in all tissues expressed. Both types of transcripts were relatively abundant in gastrointestinal tracts, liver, and pancreas (unpublished observations). These results suggest that the physiological significance of FTZ-F1β is different from that of Ad4BP which shows steroidogenic tissue-specific expression [5]. The longer form, rFTZ-F1β1, corresponded to the structure of mLRH-1 [13], whereas the shorter form, rFTZ-F1β2, corresponded to the structure of the human ortholog (unpublished observations). Judging from the complete structural identity of the two cDNAs except for the above 63 bp sequences in the A/B domain, both rFTZ-F1β1 and β2 transcripts are probably derived from the same gene by alternative splicing.

Fig. 3 shows the comparison of the cDNA structures between rFTZ-F1β1 and rat Ad4BP/SF-1 [16]. The length of the A/B domain was a striking contrast, since rat Ad4BP is only 12 amino acids long, while rFTZ-F1β1 is 106 amino acids long in the domain. The coding sequence of rFTZ-F1β1 cDNA was 60% identical to that of rat Ad4BP cDNA. The sequence comparison of each functional domain, DNA binding domain, FTZ-F1 box, region II and region III between rat FTZ-F1β1 cDNA and rat Ad4BP, revealed 85, 97, 71 and 70% homologies, respectively, thus indicating the DNA binding domain and FTZ-F1 box to be highly homologous with each other.

Since FTZ-F1β is reported to be a fetoprotein transcription factor, the tissue expression of the FTZ-F1β mRNA in heaptocellular carcinoma (HCC) and cholagiocellular carcinoma (CCC) obtained from patients at surgery was examined by reverse transcriptase polymerase chain reaction (RT-PCR). As shown in Fig. 4, regardless of the levels of AFP produced from the tumors, the expression levels of FTZ-F1β are not essentially different. These results suggest that FTZ-F1β (FTF) alone does not determine the expression level of AFP but rather works as a liver-constitutive factor for the expression of AFP. Actually, it is reported that FTF-mediated transcription of AFP gene is strongly enhanced by the presence of other transcriptional factors such as HNF-1 in vitro [13]. Further studies are needed to elucidate the physiological significance of this unique nuclear receptor.

Acknowledgements

We wish to thank Drs T. Yoshimi and T. Oki in Japan, and Dr S. Srivuthana in Thailand, for providing us with the genomic DNA of patients 4, 5 and 6, respectively.

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