

Regulation of Human *ADH/FDH* Gene Expression and Isolation of a Novel POZ
Domain Transcription Factor

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The human *ADH5/FDH* has a GC-rich promoter with many sites at which transcription factors bind. A minimal promoter extending from bp 334 to bp +61 direct high levels of transcription in several different cells, consistent with the ubiquitous expression of the gene. Nearly the entire minimal promoter can be bound by Sp1. We analyzed the transcriptional regulation of *ADH5/FDH* by member of Sp1 multigene family. Two core *cis*-elements (bp 22 to bp +22) had the highest affinity for Sp1. The zinc-finger domains of Sp3 and Sp4 also bind selectively to the core *cis*-elements. In SL2 cells, which lack endogenous Sp1, potently activated transcription, suggesting that Sp1 is critical in the initiation of transcription. Neither Sp3 nor Sp4 was able to activate transcription in those cells, and transcriptional activation by Sp1 was repressed by Sp3 or Sp4. The content of Sp1, Sp3, and Sp4 in different cells may be critical factors regulating transcription of *ADH5/FDH* gene.

We also found the human *ADH5/FDH* gene can be regulated by structural features within 5' UTR at the translational level. The 5' UTR is GC-rich and contains an uORF with two possible AUG start codons, a structural feature uncommon in mammalian genes. Mutation of either AUG codon increased reporter gene expression two folds, demonstrating an inhibitory role. Toeprint analysis demonstrated many stalled ribosomes flanking the upstream AUG codons and the presence of secondary structures near the upstream AUGs. Secondary structures may increase the ability of ribosomes to recognize the two upstream AUGs, despite their poor initiation context. The upstream ORF, presence of secondary structures, and short intercistronic distance (2 bp) between the uORF and main ORF are important translational regulatory elements of *ADH5/FDH* expression.

We isolated a novel transcription factor recognizing the bp 38 to bp 22 element located just outside of the core-*cis* elements by yeast one-hybrid system. The cDNA is very rich in GC bases (72%) and encodes a polypeptide of 585 a.a. It contains a POZ domain, 4 zinc fingers and nuclear localization signal.