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SCREENING FOR TRANSCRIPTIONAL FACTORS THAT
REGULATE THE HUMAN CLASS I ALCOHOL DEHYDROGENASE
GENES AND

CLONING AND CHARACTERIZATION OF A RAS-RELATED
TRANSCRIPTION FACTOR, LZ321

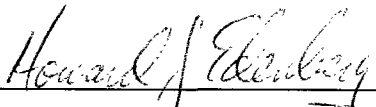
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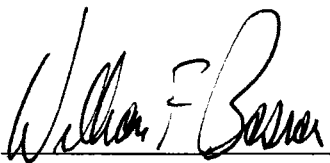
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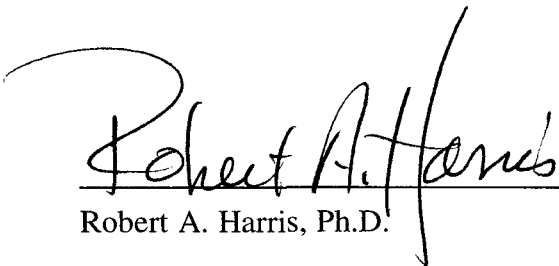
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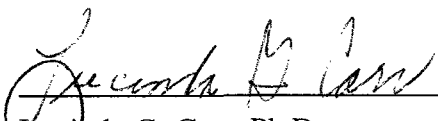
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ABSTRACT

The primary goal of my research is to study the regulation of class I alcohol dehydrogenase (*ADH*) genes. Humans have three closely related class I alcohol dehydrogenase genes, *ADH1*, *ADH2* and *ADH3*. Their proximal promoters are 80-84% identical in sequence. All three genes are highly expressed in adult liver, but they differ in both temporal expression and extent of expression in non-hepatic tissues. These properties make them good candidates for studies of gene regulation.


In an effort to search for transcription factors involved in *ADH* gene expression, we screened a human liver cDNA expression library using four trimers of *cis*-acting elements as ligands. Two positive clones were obtained. One binds to a putative HNF-1 site in *ADH2*. Competition studies showed that the binding to this ligand was sequence specific. DNA sequencing analysis identified it as a high mobility group protein, HMG-I. This result suggests that HMG-I could participate in regulating Class I *ADH* gene promoters.

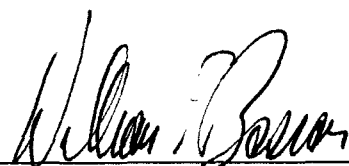
The other positive clone we isolated, LZ321, encodes a C₂H₂ type zinc-finger protein. LZ321 bound specifically to the probe sequence, but not to the original *cis*-acting element, site H in *ADH3*; it bound to a sequence created in part by the junction between two site H oligonucleotides. A trimer of this site shows cell-specific enhancer activity, increasing transcription dramatically in CV-1 cells (126-fold in one direction and 24-fold in the other direction) but only moderately in H4IIE-C3 cells (7-fold in one direction and 4-fold in the other). The LZ321 sequence matched RREB1, a potential Ras downstream transcription factor reported after our initial characterization. However, the ligand we used, that contains a *Drosophila* tkk protein consensus binding site (GGTCCT), was very different from both the original Ras responsive element (RRE) and the consensus binding site determined for RREB1. We therefore examined the binding affinities of RREB1/LZ321 to three ligands. It bound to our ligand and the RRE with similar affinities (K_d 49 nM and 60 nM), but did not bind to the reported RREB1 consensus binding site.

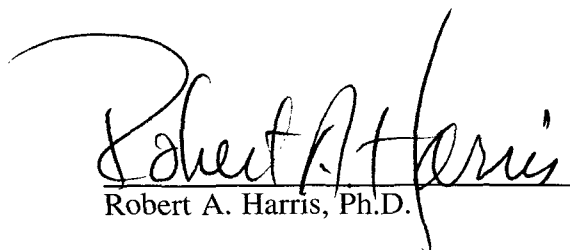
The RREB1/LZ321 protein contains four C₂H₂ type zinc-fingers. We examined the binding of truncated forms of RREB1/LZ321 to determine whether the different sequences were bound at different sites. Peptides lacking one or two N-terminal zinc-fingers retained specific DNA binding abilities to both ligands, although binding affinities decreased significantly (K_d values increased approximately 3 fold).

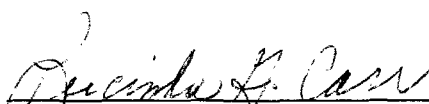
Co-transfection of a constitutively active Raf construct along with the RREB1 expression vector further stimulated transactivation of promoters with the GGTCCT sequence, demonstrating that RREB1/LZ321 could function as a downstream activator in the Ras-Raf signaling pathway, acting through this ttk-like site. These data demonstrate that RREB1/LZ321 binds to multiple different *cis*-acting elements and thereby may regulate a wide range of genes.

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