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Promoter paper

Characterization of the rat LDL receptor 5′-flanking region

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Abstract

A 1.5-kb genomic DNA fragment corresponding to the 5′-flanking region of the rat LDL receptor gene was cloned and putative regulatory regions were identified. A major transcription start site was identified at −154 bp relative to the ATG translation initiation codon, within a region containing two thyroid hormone response element half-site motifs (2H-TRE). Binding of thyroid hormone receptors α and β1 to this element was demonstrated. Mutations within this 2H-TRE region abolished basal transcription levels of the rat LDL receptor gene. Reporter gene studies indicated that the promoter region between −300 and −200 bp, which contains one sterol response element (SRE) and two specificity protein-1 sites (Sp1) sites, is crucial for basal transcription of the rat LDL receptor gene. The functionality of the SRE motif was confirmed using electrophoretic mobility shift assays and reporter gene studies.

Keywords: Promoter; Response element; Transcription start site; Rapid amplification of cDNA end; Thyroid hormone; SREBP

1. Introduction

The hepatic low density lipoprotein (LDL) receptor plays a critical role in maintaining cholesterol homeostasis by removing highly atherogenic LDL particles from the circulation [1,2]. The LDL receptor pathway mediates at least 60–75% of LDL turnover in rats [3,4], 67% in rabbits [5], and 56–80% in man [4]. The liver contains about 70% of total LDL receptors present in the body [6]; therefore, changes in plasma LDL levels are generally due to changes in hepatic LDL receptor activity. The identification of mutations in either the gene encoding the LDL receptor, the gene encoding the liver specific LDL receptor adaptor autosomal recessive hypercholesterolemia (ARH) protein, the gene encoding the protease, proprotein convertase subtilisin kexin 9 (PCSK9), or genes encoding the ligands of the LDL receptor-apolipoprotein (apo) E and apo B-100 [1,7–10], has established the importance of the LDL receptor to cardiovascular physiology of humans. Thus, a greater understanding of the regulatory mechanisms that control expression of the hepatic LDL receptor is essential.

Several animal models have been used thus far to study cholesterol metabolism in vivo. These include the monkey, bovine, rabbit, hamster, mouse, and rat [11–14]. In fact, LDL receptor cDNA clones for these species and the human have been reported [15–20]. In addition, the gene encoding the human, hamster, and mouse LDL receptor including their 5′-flanking regions have been isolated and characterized [20–22]. In the current study, we present an analysis of the 5′-flanking region of the rat LDL receptor gene. This animal model has been shown to be very useful in studying hormonal regulation because a well-defined hormone deficient state can be easily obtained without undue stress to the animal [23–26].

2. Materials and methods

2.1. Animals

Normal male Sprague–Dawley rats weighing 125 to 150 grams were purchased from Harlan Industries (Madison, WI). Animals were housed in a light-controlled reversed cycle room with 12 h of light followed by 12 h of darkness and received Purina Rodent Laboratory Chow 5001 ad libitum. Rats were euthanized at the fourth hour of the dark period by an isoflurane overdose. Liver portions were quickly removed for preparation of RNA.

2.2. Materials

Primers and oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, IA). Advantage Tth Polymerase Mix and the PromoterFinder DNA Walking Kit were purchased from Clontech Laboratories,

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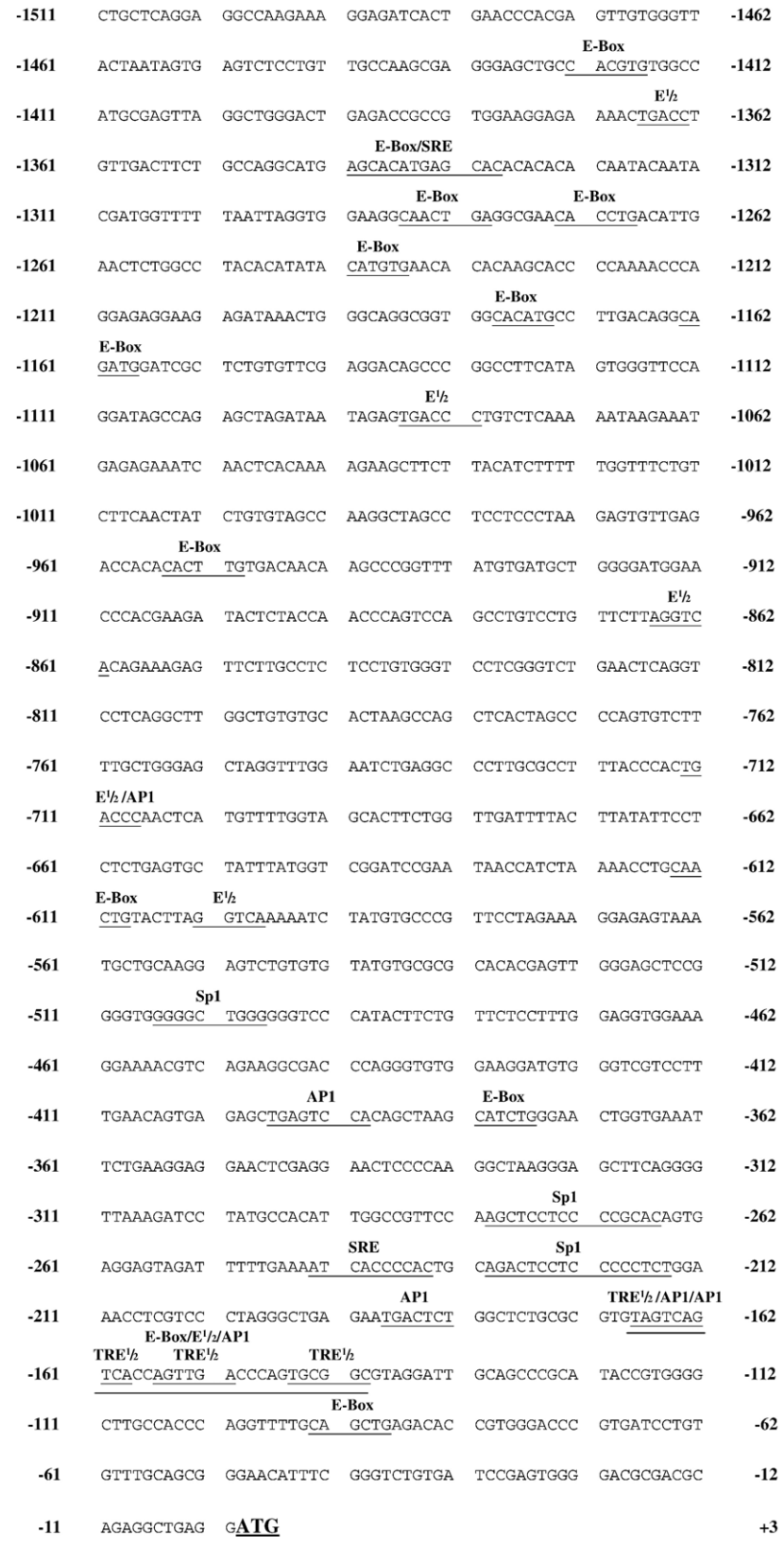

Fig. 1. Nucleotide sequence of the 5′-flanking region of the rat LDL receptor gene. Nucleotide position + 1 was assigned to the "A" of the ATG translation start codon (boldfaced-underlined). Negative numbers refer to the nucleotide region positioned to the 5′-side of the start codon. Putative E-box, estrogen response element half-site (E1/2), sterol-response element (SRE), specificity protein-1 (Sp1), adaptor protein-1 (AP1), and thyroid hormone response element half-site (TRE1/2) motifs are underlined. The rat LDL receptor 5′-flanking region has been deposited in GenBank with accession number AY675581.