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5' flanking sequence of the gene for rat hepatic cytochrome P450e

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Phenobarbital treatment of rats leads to a marked increase in hepatic levels of two closely related forms of cytochrome P450, P450b and e. To study the molecular mechanism of this induction, a genomic clone containing the 5' portion of the P450 e gene and approximately 6 Kb of flanking region was isolated from a lambda Charon 4A genomic library obtained from Dr. T. Sargent (NIH, Bethesda, MD) by the in vivo recombination screening procedure of Seed (1) using a fragment from a previously described P450e genomic clone (2) encompassing exon 2 and short flanking intronic regions as a probe. A subcloned fragment extending from the NcoI site at the initiation codon to the Hind III site approximately 1.4 Kb upstream from the cap site was used to prepare a library of random DNA subfragments for sequencing by the dideoxy method (3). This fragment was found to contain 1406 base pairs of 5' flanking region and 27 bases of the first exon. Sequence analysis showed that P450 e gene has a modified TATA sequence, CATAAA, 20 bases upstream from the transcription initiation site (4) and an alternating purine- pyrimidine CA repeated nineteen times at about 255 bases upstream from the cap site. This repeated sequence is capable of forming a Z-DNA structure, which may play a role in the regulation of gene expression (5). Analysis of the sequence also showed the presence of a gluco-corticoid receptor binding site (GRE) between bases -1339 to -1357 in a similar arrangement as reported recently for the tyrosine aminotransferase gene and other glucocorticoid regulated genes (6). The presence of this binding site may be related to the recently observed dramatic increases in the P450 b and e proteins in livers of dexamethasone treated rats (7). A small portion of the rat P450 e gene 5' flanking sequence with the transcription initiation site has been previously reported (4).

-1406
AAGCTTTTCCTCTAAGTGTCCTGCCACCCCCCACCCCAATAATATCAGTTAGGGTACAAAGTGTTCAAACATGAACTTCTGAGACAGA
TTTCACATTCAAATAGAACACATTATGAATAGATTAATGTTATCACTGTAGTGGTGGGCTGGTGAAGAATGTTCAATTCCTTTTAGCA
AGATGGAAGGTCAAAGAACTTCCTGTGCTATGAACAAATCAGAAGGATGAAGGGAACCATTTGTCATTAGACACAGTGTTCAGAGACT
ATCTTTGTTAGGTTCACTATTTCTGTGATAAAACCTCACAGCAGAAAGCAACTTGGGGAAAAGGGTTTATTTTGCTTGCACTTTACAG
TTTGTCAAACAAGGAAGTCAGGGCACGAAGACAAGGCAGGACCTGGAGGCAGGAGCTGATGCAGAGGCCATGGAGGGATGCTTCTCAC
TGACTTGCTCATTAGGGCTTGCTCAGCCTGATTTCTTACAGAACCCAAGACTTTCTTACAGAAGTCCAGGTGTGGAATTTCCCACAGT
GGGTGGTGCCCTCCCTCATGCAATCACTAATTAAGAAAATGCTCTTCAGGCTTGCGTGTAGTGTGATCTTATGGATCCATTTTCTCAA
TTCAAGTTCTCTCCTCTCAAATGACTCTAGCTTGTATCATGTTGACACAAAAGTTAGACCCGGGGCCCCAACTTCTTCTCTGCCAGGT
TTGAGCCTCTCTCTCCATTTGACTCCTGAGCCTCACTGCTCCTGAATCTCTTGCCTTTCCTTTCTTTTCCTCCCCATTCTCCCACCTG
TGGCTATCATGGGCCAGAGAGTGAAATGGGGACTCTGCAGAAATTGCATCTGTAGACTTTTCAAAGACAGGGACAGGAACCAACAGAC
GGAGACAAGCACAGGATCATGGGATACTATTCTTGTCAACTCAAACATAATCACATGTACCCAGGACACAAAAAACATACAGAGAAGC
CCCAATAATTTAAGATTATACATGTAAATATACCCTAGACATGCAAGAAAAGACCACCCAGTGCATCTAGACTCAGACAAAGAAATTT
ACATCGGTACGTTTATATCAGAAATGATCTTTCACATAGGAAAAGCATATAGAACACGCACACACACACACACACACACACACACA
CACACACAATCCCATGCCCTAGTAAGTAAACAGAGCTGACAAAACTGAGTTGACAAGTGCACACCCATTTACATAAAACAAGAGGCCT
AAGTCCCAGTGCCCTTTTGTCCTGTGTATCTGTTTCGTGGTGTCCTTGCCAACATCTATGGTGTGGGTAAGGGAATGAGGAGTGAATA
GCCAAAGCAGGAGGCGTGAACATCTGAAGTTGCATAACTGAGTGTAGGGGCAGATTCAGCATAAAAGATCCTGCTGGAGAGCATGCAC
TGAAGTCTACCGTGGTTACACCAGG

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