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TISSUE DISTRIBUTION AND TRANSCRIPTIONAL REGULATION OF  
MITOCHONDRIAL ALDEHYDE DEHYDROGENASE

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Submitted to the faculty of the University Graduate School  
in partial fulfillment of the requirements for the degree


Doctor of Philosophy


in the Department of Biochemistry and Molecular Biology

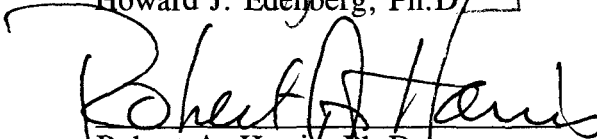
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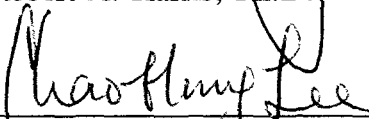
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## ABSTRACT

### TISSUE DISTRIBUTION AND TRANSCRIPTIONAL REGULATION OF MITOCHONDRIAL ALDEHYDE DEHYDROGENASE

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The acetaldehyde that is generated during alcohol metabolism is oxidized to acetate by mitochondrial aldehyde dehydrogenase (ALDH2). Acetaldehyde is a very toxic substance and is the causative agent in the Asian alcohol flush reaction. The flush reaction is due to a mutation in ALDH2; however, there is a large amount of variation in the degree of flushing between individuals. This variation may be explained by differences in the level of transcription of the *ALDH2* gene. This work investigates the tissue distribution of ALDH2 by examination of ALDH2 activity, and levels of protein and mRNA in various rat tissues. These studies showed that ALDH2 activity, immunoreactive protein, and mRNA were at highest levels in liver and at intermediate levels in kidney and lung. Because the production of mRNA and protein are most often controlled at the level of transcription of the gene, the regions of the *ALDH2* 5' flanking sequences were examined. The 5' flanking region of the *ALDH2* promoter is very rich in guanosine and cytosine residues. These nucleotides were predominantly unmethylated in the tissues examined as determined by digestion with MspI and HpaII and Southern

blot analysis. Gel retardation assays and DNaseI footprint analysis were performed on the *ALDH2* promoter to determine regions bound by nuclear proteins. The functional importance of these regions was assessed by transient transfections of *ALDH2* sequences driving the transcription of a reporter gene. DNA binding studies revealed that the ubiquitous transcription factor nuclear factor Y/CCAAT-binding protein 1 (NF-Y/CP1) bound to the proximal *ALDH2* promoter. Deletion of this region abolished transcription of the reporter gene in transient transfection assays. The further 5' region was bound by a novel liver-specific factor (LF1). These data suggest that the proximal promoter is bound by NF-Y/CP1 which may be important for the levels of expression seen in kidney and lung. The liver specific expression of *ALDH2* appears to be modulated in part by binding of a novel liver specific factor to the distal *ALDH2* promoter.

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