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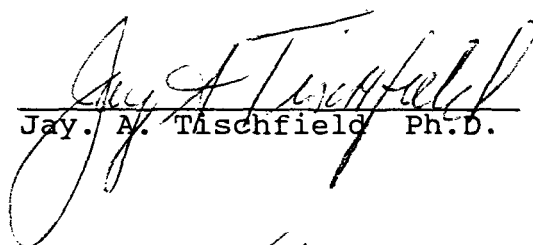
**MOLECULAR ANALYSIS OF FORWARD AND REVERSE SOMATIC
MUTATIONS AT THE HUMAN APRT LOCUS**

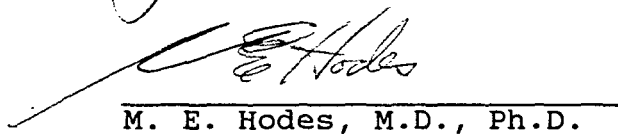
Yuan Zhu

**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 Medical and Molecular Genetics
Indiana University**

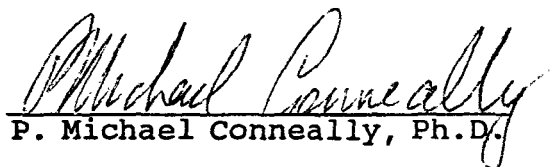
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June 17, 1992

ABSTRACT

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MOLECULAR ANALYSIS OF FORWARD AND REVERSE SOMATIC MUTATIONS AT THE HUMAN APRT LOCUS

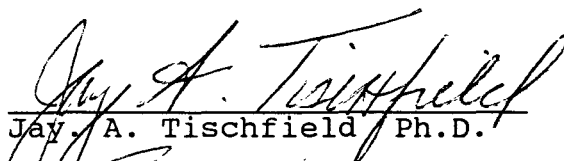
Somatic cell mutation plays an important role in the etiology of a number of genetic diseases and many types of cancer, e.g. retinoblastoma and Wilms tumor. This thesis describes the use of the human adenine phosphoribosyltransferase (APRT) gene in cell culture for study of the nature and consequences of spontaneous and induced mutations at a typical mammalian locus.

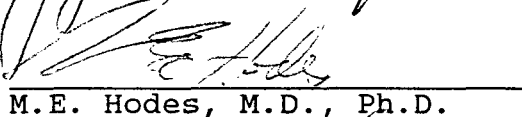
Two APRT-deficient human cell lines were analyzed. An ICR170H-induced fibrosarcoma cell line, HTD114, was shown to have a single G insertion in an existing string of 5G's in exon 2 of one allele and in exon 3 of the second allele. A hepatoma cell line, 3B₂25H, was shown to have a G → C transversion at the third base pair of intron 1, which results in very low levels of normal APRT mRNA.

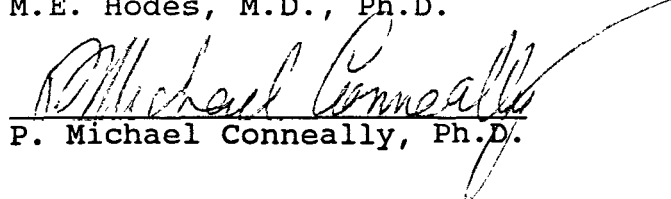
With an APRT reversion assay in HTD114 cells, mutagens and carcinogens, such as mitomycin C, benzo[a]pyrene diol epoxide, 2-aminoanthracene, 7,12-dimethylbenz[a]anthracene and aflatoxin B₁ were demonstrated to induce a single base-pair deletion that resulted in frame restoration. The APRT reversion rates induced by these chemicals except for mitomycin C ($1.2 - 3.3 \times 10^{-5}$) are about 10^3 fold increased over the rate of spontaneous reversion. Rat liver homogenate coupled with NADPH cofactors was an essential activation

system for some mutagenesis. However, mitomycin C and other mutagens produced little effect on the induction of interallelic mitotic recombination.

By selection and analysis of Aprt^r mutants in a human cell line heterozygous at APRT (APRT^{+/-}), the molecular mechanisms of loss of APRT activity were investigated. These included loss of the wild-type allele (62%) and intragenic mutations (38%). Loss of the wild-type APRT allele was frequently accompanied by loss of the relatively close proximal marker D16S77, but not the more distant proximal marker D16S4. These data indicate that high frequency mitotic recombination or deletion occurred at the region between D16S77 and D16S4 on chromosome 16. Also, point mutations were demonstrated to be responsible for the loss of APRT activity in other clones. These demonstrated mechanisms for expression of a recessive phenotype at an autosomal locus are similar to those found in retinoblastoma and other tumors. Thus, APRT may be used to model loci important to carcinogenesis.


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