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THE CASEIN KINASE I FAMILY OF ENZYMES

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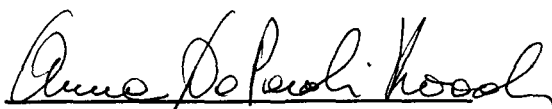
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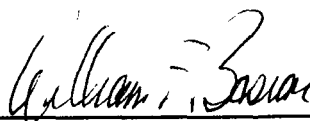


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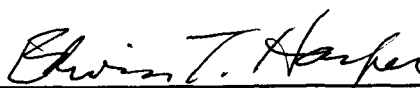


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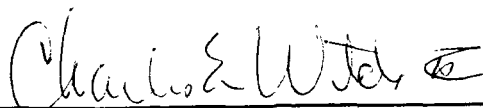
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ABSTRACT

Casein kinase I (CKI) is a ubiquitous protein serine and threonine kinase known to phosphorylate a wide variety of proteins. However, for many years, very little was known about the enzyme itself and in 1990 there was still no amino acid sequence available. Therefore, we pursued the molecular cloning of the enzyme. We obtained a cDNA clone for CKI and from its amino acid sequence, were able to identify several yeast CKI homologs. Currently, seven distinct mammalian CKI isoforms and eight yeast enzymes have been identified by molecular cloning. These isoforms form one of the largest protein kinase subfamilies yet discovered and are a distinct branch of the protein kinase tree. From the deduced amino acid sequence we compared our particular cDNA clone (CKI δ), to other CKI isoforms. Interestingly, it showed high identity to three yeast CKI homologs shown to be involved in DNA repair termed Hhp1, Hhp2 and Hrr25p. In addition, CKI δ shows similarity to nuclear forms of CKI and contains a putative nuclear localization signal. Finally, its message level was most abundant in testis. Thus, CKI δ may be a nuclear enzyme involved in DNA repair processes. Expression of the CKI δ cDNA in *Escherichia coli* resulted in active enzyme that resembled native CKI. Further study revealed recombinant enzyme to be regulated by two interrelated processes. First, CKI δ underwent extensive autophosphorylation in *E. coli* and could be activated by protein phosphatase. Second, CKI δ could be activated by removal of the C-terminal non-catalytic domain. This truncated form of the enzyme was not activated by phosphatase. Analysis of additional truncations localized the inhibitory domain between residues 317-343 which contained six potential

autophosphorylation sites. Thus, CKI δ contains an autoinhibitory domain located in its C-terminal region which becomes inhibitory when it is phosphorylated. These mechanisms of enzyme regulation could have important consequences *in vivo*.

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