Vol. 203, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1994 Pages 231-236

MOLECULAR CLONING AND SEQUENCE ANALYSIS OF TWO NOVEL FISSION YEAST CASEIN KINASE-1 ISOFORMS

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Received June 22, 1994

The cDNAs for two casein kinase-1 homologs, *hhp1* and *hhp2*, have been isolated from *Schizosaccharomyces pombe* and characterized. Their corresponding genes reside on chromosomes II and I, respectively, and encode ≈42-46 kDa proteins that are related structurally to the *HRR25* gene product of budding yeast. On the basis of multiple sequence alignment, the CK1 family appears to consist of three main branches. We predict that the branch containing the *hhp* genes encodes nuclear kinases involved in the regulation of DNA metabolism.

Casein kinase-1 (CK1) is a protein kinase common to all eukaryotic cells (1). Once considered a single entity, it is now known to consist of subspecies that together comprise a distinct branch of the eukaryotic protein kinase family (2, 3, 4, 5). Family members identified to date consist of a highly conserved, ≈290 residue N-terminal catalytic domain, joined to a C-terminal region that is poorly conserved among family members and that varies in size from 40 to 180 amino acids. Although the role of the C-terminal domain is not understood fully, in some CK1 homologs it functions to target the enzyme to specific regions of the cell (3).

The identification of CK1 family members in simple eukaryotes such as budding yeast as well as in mammals (2, 6) and higher plants (7) suggests these enzymes play an important and possibly conserved role in cell regulation. The situation is complicated, however, by the existence of multiple CK1-like enzymes that appear to have non-overlapping functions. In budding yeast, for example, three CK1 homologs have been identified (3, 5, 8). Two of these, *YCK1* and *YCK2*, supply an activity that is essential for vegetative growth, and when overexpressed, confer halotolerance (5). A third CK1 homolog, encoded by *HRR25*, is a regulator of DNA metabolism (4) and supplies an activity that is not complemented by either *YCK* gene.

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0006-291X/94 $5.00

To develop an additional system for study of CK1 function, we initiated a search for CK1 homologs in the fission yeast *Schizosaccharomyces pombe*. Four CK1-related genes were identified in this organism. The first two genes, termed *cki1*$^+$ and *cki2*$^+$, emerged as homologs of the *YCK* genes of budding yeast, and have been described in detail (9). Here we describe the remaining two CK1-related genes, termed *hhp1*$^+$ and *hhp2*$^+$, the products of which appear to be the homologs of budding yeast Hrr25p and mammalian CKIδ.

MATERIALS AND METHODS

DNA manipulation and analysis
Cloned cDNAs were obtained from an *S. pombe* cDNA library constructed in lambda-ZAPII (10), isolated as *Not*I fragments, and subcloned into pSK(-)ΔH. These and other general molecular biology methods were performed as described previously (3, 9).
Physical mapping
The chromosomal locations of *hhp1* and *hhp2* were established by hybridizing their nick-translated full-length cDNAs to filters containing overlapping members of the *S. pombe* genomic library described by Mizukami *et al.* (11).
Sequence analysis
The amino acid sequences of CK1 isoforms were aligned and displayed in graphical form as described by Higgins and Sharp (12, 13).

RESULTS AND DISCUSSION

Isolation and chromosomal location of CK1 homologs in fission yeast.
Two fission yeast CK1 homologs were identified by low stringency genomic Southern analysis using an 859-bp *Nla*IV fragment encoding the catalytic core of *HRR25* as a probe. Hybridization of the same probe to a lambda phage-based cDNA library led to the isolation of two cDNAs with open reading frames homologous to CK1. Their corresponding genes were named *hhp1*$^+$ and *hhp2*$^+$ (*HRR25* homolog in pombe). High stringency genomic Southern analysis using either *hhp1* or *hhp2* cDNA as probe confirmed that these genes were distinct from *cki1* and *cki2* (9).

Full-length nucleotide sequences of *hhp1* and *hhp2* along with the predicted amino acid sequences of their products are shown in Figs. 1 and 2, respectively. The longest open reading frame of *hhp1* encodes a protein of 365 amino acids, (calculated molecular mass and isoelectric point of 42,450 and 9.7, respectively) whereas *hhp2* encodes a protein of 399 amino acids (calculated molecular mass and isoelectric point of 45,757 and 9.8, respectively).

The location of *hhp1*$^+$ and *hhp2*$^+$ in the *S. pombe* genome was determined as described in Materials and Methods. Hybridization to cosmid clones 170 and 1821 on contig 5 (position 263) placed *hhp1*$^+$ on right arm of chromosome II, approximately 270 kb from the centromere and between *ran1*$^+$ and *leu1*$^+$ on the physical map. Hybridization to cosmid clones 139 and 968 on contig 3 (position 38) placed *hhp2*$^+$ on the left arm of chromosome I, approximately 1.1 Mb from the left

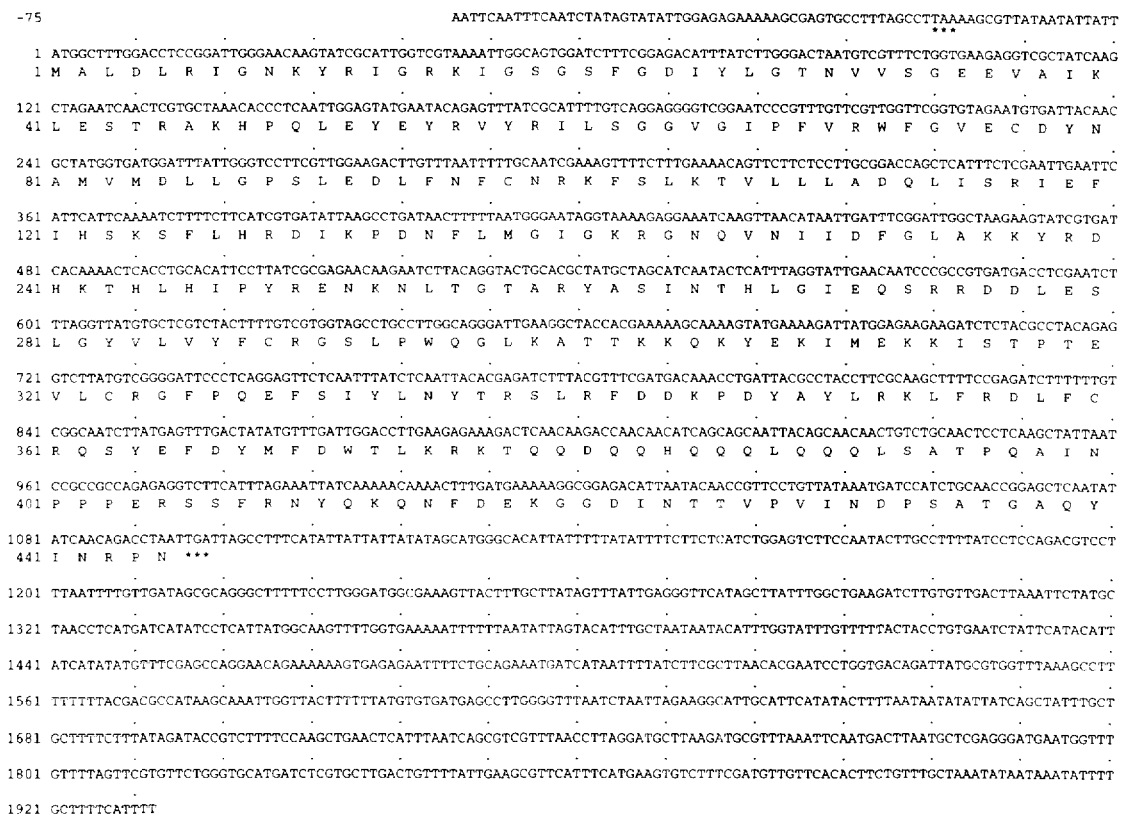

Fig. 1. Nucleotide (cDNA) and deduced amino acid sequences of *hhp1*. Numbering of nucleotides and amino acid residues begins with the first methionine codon of the open reading frame. Asterisks mark in-frame stop codons.

telomere and between *cut5*+ and *csk1*+ on the physical map. Although three of the four known fission yeast CK1 genes (*cki1*+, *cki2*+, and *hhp2*+) are found on chromosome II (9), they are separated by at least 1 Mb and are not closely linked.

Predicted amino acid sequences of hhp1 and hhp2.

The deduced primary structures of hhp1 and hhp2 retain the conserved structural organization observed for other CK1 isoforms, including a highly conserved N-terminal catalytic domain that lacks the common peptide triplet Ala-Pro-Glu in subdomain VIII. The absence of the third residue of this triplet (Glu) is unique among protein kinases and is diagnostic for CK1 family members.

Following the catalytic domain is a hydrophilic, 60 - 95 residue segment that is poorly conserved between the two enzymes and that shares little sequence similarity with analogous regions of the *YCK* or *cki* gene products (3, 9). Neither sequence contains consensus sequences for prenylation that are common to the cytoplasmic yeast CK1 isoforms (9).

To determine their phylogenetic relationship, ten CK1 homologs from fission yeast, budding yeast, and mammalian species were subjected to multiple amino

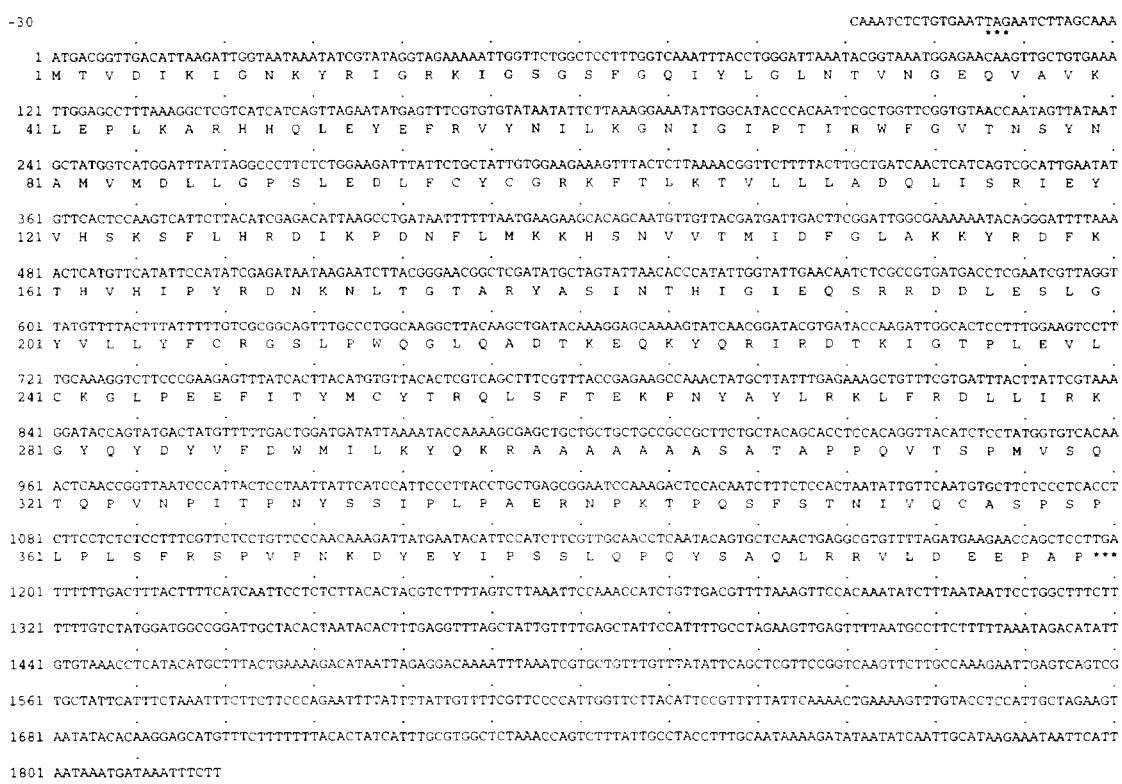

Fig. 2. Nucleotide (cDNA) and deduced amino acid sequences of *hhp2*. The Numbering of nucleotides and identification of stop codons are as in Fig. 1.

acid sequence alignment, and ordered on the basis of pairwise similarity as described in Materials and Methods. The resultant dendrogram reveals that the CK1 family clusters into three main branches (Fig. 3). The first of these consists of the yeast *YCK* and *cki* gene products. These enzymes are found exclusively in the cytoplasm, where they associate primarily with the plasma membrane (9, 14) and participate in the control of cell morphology (9, 15). Although a mammalian homolog of these enzymes has not been identified, we note that the partial sequence of CKIγ (2) is more closely related to this branch of the CK1 family than those described below.

The second dendrogram branch is exemplified by Hrr25p. This enzyme is found largely in the nucleus (14), where it regulates double-strand break DNA repair pathways (4, 8). On the basis of sequence homology, we predict that the fission yeast CK1 homologs identified here, and mammalian CKIδ, are homologs of Hrr25p. Indeed, CKIδ resembles the nuclear form of mammalian CK1 (6, 16).

The final branch consists of the classical, ≈37-kDa mammalian isoforms, CKIα and CKIβ. Although their catalytic domains are very similar to those of the Hrr25p branch (≈70% identical amino acid sequence), they appear to distribute broadly

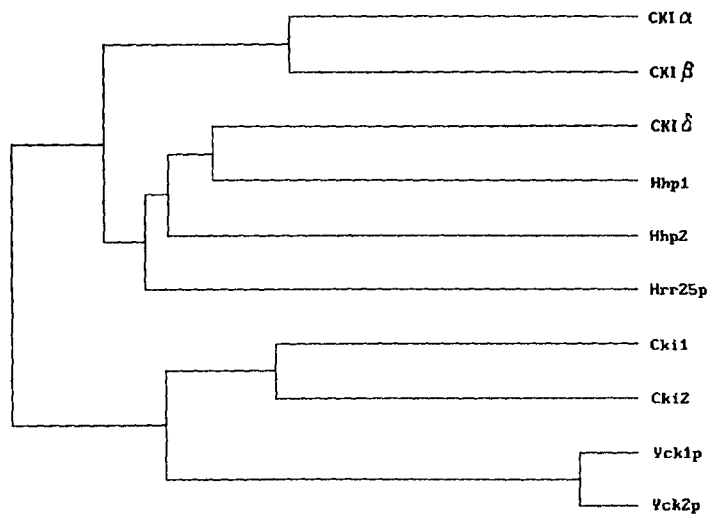

Fig. 3. Dendrogram of ten CK1 isoforms. Full-length sequences of budding yeast (Yck1p, Yck2p, Hrr25p), fission yeast (Cki1, Cki2, Hhp1, Hhp2), and mammalian (CKIα, β, and δ) CK1 isoforms (2, 3, 5, 6, 9) were aligned and displayed as described in Materials and Methods. Branch lengths are not to scale.

throughout the cell, where they may perform multiple functions. For example, CKIα shows cell cycle-dependent localization to mitotic spindles (17), implicating the enzyme in mitosis. Similar forms of mammalian CK1 are activated by insulin, interleukin-1, and Tumor Necrosis Factor in a dose-dependent fashion (18, 19, 20), suggesting a role in signal transduction. A structural homolog of this branch is not apparent in yeast cells.

In summary, the primary structures of Hhp1 and Hhp2 reveals them to be conserved members of "Hrr25p" branch of the CK1 family of kinases. The close structural relationship with Hrr25p suggests these enzymes participate in the regulation of DNA repair mechanisms. The genetically tractable CK1 homologs of fission yeast will prove useful in testing this hypothesis, and correlating structure (21) with *in vivo* function.

ACKNOWLEDGMENTS

We thank David Beach and Nancy Kaplan for mapping data, and Spencer Teplin for oligonucleotide synthesis. Nucleotide sequence data reported herein appear in the GenBank/EMBL nucleotide sequence databases under accession numbers U10863 (hhp1) and U10864 (hhp2). This work was supported by a grant from the National Institutes of Health (GM 42816).

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