THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 270, No. 25, Issue of June 23, pp. 14875–14883, 1995
Printed in U.S.A.

Isolation and Characterization of Human Casein Kinase Iε (CKI), a Novel Member of the CKI Gene Family*

(Received for publication, January 23, 1995, and in revised form, March 29, 1995)

Kimberly J. Fish‡§, Aleksandra Cegielska‡§, Michael E. Getman¶‖, Gregory M. Landes¶, and David M. Virshup‡§‡‡**

*From the ‡Program in Human Molecular Biology and Genetics, §Department of Oncological Science, Division of Molecular Biology and Genetics, and **Department of Pediatrics, University of Utah, Salt Lake City, Utah 84112, ¶Department of Human Genetics, Integrated Genetics, Inc., Framingham, Massachusetts 01701, and the ‖Worcester Polytechnic Institute, Worcester, Massachusetts 01609*

The casein kinase I (CKI) gene family is a rapidly enlarging group whose members have been implicated in the control of cytoplasmic and nuclear processes, including DNA replication and repair. We report here the cloning and characterization of a novel isoform of CKI from a human placental cDNA library. The cDNA for this isoform, hCKIε, predicts a basic polypeptide of 416 amino acids and a molecular mass of 47.3 kDa. It encodes a core kinase domain of 285 amino acids and a carboxyl-terminal tail of 123 amino acids. The kinase domain is 53–98% identical to the kinase domains of other CKI family members and is most closely related to the δ isoform. Localization of the hCKIε gene to chromosome 22q12–13 and the hCKIδ gene to chromosome 17q25 confirms that these are distinct genes in the CKI family. Northern blot analysis shows that hCKIε is expressed in multiple human cell lines. Recombinant hCKIε is an active enzyme that phosphorylates known CKI substrates including a CKI-specific peptide substrate and is inhibited by CKI-7, a CKI-specific inhibitor. A budding yeast isoform of CKI, HRR25, has been implicated in DNA repair responses. Expression of hCKIε but not hCKIα rescued the slow-growth phenotype of a *Saccharomyces cerevisiae* strain with a deletion of HRR25. Human CKIε is a novel CKI isoform with properties that overlap those of previously described CKI isoforms.

Casein kinase I (CKI)[1] was among the first serine/threonine protein kinases described. CKI is a ubiquitous monomeric enzyme ranging in size from 25 to 55 kDa and is present in membranes, nucleus, cytoplasm, and cytoskeleton of eukaryotic cells, and on mitotic spindles of mammalian cells (Tuazon and Traugh, 1991; Brockman *et al.*, 1992). Its substrate specificity is determined both by acidic or phosphoryl groups three to four residues amino-terminal to the target residue (Flotow *et al.*, 1990; Flotow and Roach, 1991), and by the tertiary structure of the substrate (Cegielska *et al.*, 1994). Protein substrates for CKI identified *in vitro* encompass both nuclear and cytosolic proteins including glycogen synthase, RNA polymerases I and II, p53, and simian virus 40 (SV40) large T antigen. However, in only a few instances has phosphorylation by CKI been shown to correlate with changes in function of the substrate. For example, phosphorylation of large T antigen by CKI inhibits its ability to initiate viral DNA replication (Cegielska and Virshup, 1993; Cegielska *et al.*, 1994). Similarly, glycogen synthase activity is significantly inhibited by synergistic phosphorylation by cyclic AMP-dependent protein kinase and CKI (Flotow and Roach, 1989).

cDNA cloning has revealed that CKI comprises an ever-growing family of highly related proteins. In recent years a number of isoforms of casein kinase I have been identified from a variety of sources. Four mammalian isoforms, α, β, γ, and δ, were first defined in a bovine brain cDNA library (Rowles *et al.*, 1991). A full-length δ isoform, CKIδ (both protein and cDNA), was subsequently isolated from rat testes (Graves *et al.*, 1993). In budding yeast, at least two membrane-associated isoforms, CKI1 and CKI2, are required for vegetative growth (Robinson *et al.*, 1992, 1993; Wang *et al.*, 1992). In *Schizosaccharomyces pombe*, two homologs, *cki1*$^+$ and *cki2*$^+$, encode distinct but related cytoplasmic enzymes (Wang *et al.*, 1994). A number of the yeast casein kinase I isoforms have tyrosine kinase activity (Hoekstra *et al.*, 1994).

CKI homologs in budding and fission yeast appear to regulate aspects of cellular DNA metabolism. HRR25, a *Saccharomyces cerevisiae* homolog of CKI, was identified in a screen for mutants sensitive to DNA double-strand breaks (Hoekstra *et al.*, 1991; DeMaggio *et al.*, 1992). Yeast with mutations in HRR25 grow very slowly, and exhibit sensitivity to endonuclease cleavage, alkylating agents, and x-irradiation. Cells with null mutations of HRR25 accumulate in the G_2 phase of the cell cycle. A pair of related CKI homologs, *hhp1*$^+$ and *hhp2*$^+$, play a similar role in DNA repair in *S. pombe* (Dhillon and Hoekstra, 1994).

To better understand the role of CKI in cellular metabolism we have characterized human members of the gene family. Here we report the cloning, sequence, genomic localization, and activity of a novel member of the CKI family. This isoform, which we have termed human CKI epsilon (hCKIε), is most closely related to CKIδ. Unlike CKIα, hCKIε contains a carboxyl-terminal extension of 123 amino acids that is similar in size (but not sequence) to those present in yeast CKI family members. Expression of hCKIε (but not hCKIα) rescued the slow growth phenotype of budding yeast deleted for the HRR25 gene. These findings demonstrate that human and yeast CKI family members share both sequence and functional similari-

* This research was supported in part by National Institutes of Health Grant R01 AI31657 and the Primary Children's Medical Foundation (to D. M. V.). National Cancer Institute Grant 5 P30 CA42014 subsidized a portion of the costs of oligonucleotide and peptide synthesis. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L37043, (hCKIε) and L37042 (hCKIα2).

‡‡ To whom correspondence should be addressed: Program in Human Molecular Biology and Genetics, University of Utah, Bldg. 533, Rm. 4420A, Salt Lake City, UT 84112. Internet: virshup@gene1.med.utah.edu.

[1] The abbreviations used are: hCKIε, human casein kinase Iε; bp, base pair; CKIδ, casein kinase Iδ, PAGE, polyacrylamide gel electrophoresis; kb, kilobase pair; FLpter%, fractional length from p terminus.

ties and suggest that the ε isoform may be involved in mammalian DNA metabolism.

MATERIALS AND METHODS

Molecular Cloning and Sequencing of hCKI cDNA Clones—Polymerase chain reaction primers were designed using the sequence of bovine CKIδ as a guide. Forward primer (5′-AGA CCG CGG CTG GGG AAG AAG GGC AAC TTG GT) containing a *Sac*II site and reverse primer (5′-GGA AGA TCT GGT GGG TGC GGG CGT CCC) containing a *Bgl*II site were used to amplify a 94-base pair (bp) fragment from a human placental cDNA library in λZapII (Stratagene). The polymerase chain reaction product was sequenced and used as a probe (Probe 1, Fig. 1*A*) to screen the library. Positive clones were plaque-purified, and plasmids were isolated from the recombinant phage according to the manufacturers instructions (Stratagene). The library was probed a second time with a 760-bp *Eco*RI fragment of the 10.1 (hCKIε) clone (Probe 2, Fig. 1*A*) isolated in the first screen (Fig. 1*A*). cDNAs were sequenced on both strands using the Sequenase 2.0 sequencing kit (U. S. Biochemical Corp.).

Human CKIα cDNAs were isolated from the same library using a 1.1-kilobase pair bovine CKIα cDNA clone (provided by Melanie Cobb, University of Texas, Southwestern) as a probe. One positive cDNA clone, 2.2.1, was sequenced on both strands. The insert contains a 204-nucleotide 5′-untranslated region, an open reading frame of 1011 nucleotides encoding a protein of 337 amino acids, and 170 nucleotides in the 3′-untranslated region. The deduced amino acid sequence of the 2.2.1 cDNA indicates extremely high conservation between human and bovine CKIα with 100% sequence identity at the amino acid level. It lacks the 84-bp insert found in the bovine αL form (Rowles *et al.*, 1991) but contains a novel 12-amino acid insertion at the carboxyl terminus. Because a human CKIα clone without the 12-residue insertion has been isolated previously,[2] we refer to our clone as hCKIα2. hCKIα2 appears to be an additional splice variant of CKIα. This sequence has been deposited in GenBank with accession number L37042. A cDNA clone similar to the one described here has recently been reported (Tapia *et al.*, 1994). That clone differs from our hCKIα2 clone at 27 nucleotides, 12 of which lie in the coding region and are responsible for mismatches at 4 of the amino acids predicted by the primary sequence.

Northern Blot Analysis—Total RNA was isolated from human tissue culture cell lines 293, HeLa, HL60, MCF-7, NMB, HCT116, HCT15, and DLD1 by the acid-phenol extraction method. Poly(A)-selected RNA was isolated from HeLa cells by $CsCl_2$ purification and Oligotex-dT matrix (Qiagen). RNA from both sources was separated by denaturing gel electrophoresis in 1% agarose and transferred to supported nitrocellulose (Hybond C-super, Amersham) essentially as described (Ausubel *et al.* (1994), and manufacturers instructions). The filters were probed with nick-translated ^{32}P-labeled full-length hCKIα2, hCKIδ, and hCKIε cDNAs or with a 0.9-kb *Pst*I fragment of mouse α actin cDNA.

Isolation of hCKI Genomic Clones—Genomic clones for hCKIα, hCKIδ, and hCKIε were isolated from a P1 human genomic library (Shepherd *et al.*, 1994) using the corresponding full-length cDNAs. Single clones A115C12 and E14G10 were obtained for hCKIα2 and hCKIε, respectively, while two clones, D46B1 and D76C5, were obtained for hCKIδ. By restriction mapping, P1 clones D46B1 and D76C5 both had overlapping fragments that aligned with fragments present on genomic Southern blots probed with hCKIδ. Additionally, two overlapping clones, cos3.3 and cos4.2, were isolated from a total human placental cosmid library (Stratagene) probed with hCKIε.

Fluorescent in Situ Hybridization—Metaphase spreads were prepared from normal cultured 46, XY lymphoblasts by standard procedures. Probe preparation and fluorescence *in situ* hybridization were performed essentially as described (Lichter *et al.*, 1988; Curran *et al.*, 1992). In addition, Cot1 and salmon sperm DNA were both present in the hybridization mixture at 5 μg/ml. Biotinylated probes were visualized using fluorescein isothiocyanate-avidin (5 μg/ml, Vector Laboratories, Burlingame, CA) or Cy3-steptavidin (2 μg/ml, Jackson ImmunoResearch Laboratories, West Grove, PA). Digoxigenin-labeled probes were detected using fluorescein isothiocyanate-anti-digoxigenin (0.5 μg/ml, Boehringer Mannheim). Preparations were examined using a Zeiss Axioskop microscope equipped for epifluorescence with the appropriate filter sets. Photographs were taken directly from the microscope onto Kodak Gold 400 film. Digitized images of hybridized preparations were obtained using a PXL cooled CCD camera (Photometrics, Tucson, AZ) mounted on a Zeiss Axioskop microscope. Fractional lengths (Lichter *et al.*, 1990) were determined as described previously (Landes *et al.*, 1995) except that digitized images were analyzed by a chromosome mapping algorithm developed by Biological Detection Systems (BDS, Pittsburgh, PA). For each genomic clone, 10 hybridized metaphase chromosomes were analyzed and the FLpter% determined. The high and low values were eliminated and the remaining values averaged and the standard deviation (S.D.) determined. Localizations are denoted as FLpter% ± 2 S.D.

Bacterial Expression of hCKIε—The hCKIε clone 10.1 in the pBluescript polylinker was digested with *Nco*I to completion and partially with *Sal*I. The 1333-bp product was inserted into *Nco*I/*Xho*I-digested pET16b (a T7-based expression vector (Novagen)). The pET-hCKIε expression construct (pKF115) contains 1248 bp of coding sequence followed by 55 bp of 3′-untranslated sequence and 30 bp of pBluescript sequence. This construct removes the hexahistidine sequence present in the pET16b vector downstream of the *Nco*I site. The construct was transformed into *Escherichia coli* strain BL21(DE3) pLysS, which contains the T7 RNA polymerase gene under the control of the lacUV5 promoter and a plasmid encoding T7 lysozyme (Studier *et al.*, 1990; Studier, 1991). Bacteria were grown at 37 °C in L broth containing 50 μg/ml carbenicillin and 34 μg/ml chloramphenicol to an A_{600} between 0.7 and 0.85. Isopropyl-1-thio-β-D-galactoside was then added to a final concentration of 0.4 mM and the cultures were grown for an additional 3 h at 37 °C. Cells were collected by centrifugation, snap-frozen in liquid nitrogen, and lysed by thawing on ice and resuspending in 1/30 the original volume of buffer B consisting of 20 mM HEPES, pH 7.5, 1 mM dithiothreitol, 1 mM EDTA, 10% sucrose, 0.02% Nonidet P-40, 0.1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin, with the addition of 200 μg/ml DNase I. The extracts were incubated on ice for 60 min with occasional mixing and clarified by centrifugation in a microcentrifuge at 12,000 × *g* for 15 min. The supernatants were used either directly or after immunoprecipitation for kinase assays.

Kinase reactions were performed in buffer containing 20 μM ATP, 30 mM HEPES, pH 7.5, 7 mM $MgCl_2$, 0.5 mM dithiothreitol, and 1–5 μCi of [γ-^{32}P]ATP in a final volume of 20 μl, and incubated for 30 min at 37 °C. Peptide phosphorylation reactions contained 1 mM D4 peptide (DDDDVASLPGLRRR (Flotow and Roach, 1991)) and were quantitated by spotting trichloroacetic acid-treated reactions on P81 phosphocellulose filters (Casnellie, 1991). Other reactions were stopped by the addition of SDS-PAGE sample buffer and were analyzed by SDS-PAGE and autoradiography as described previously (Cegielska and Virshup, 1993; Cegielska *et al.*, 1994).

Anti-peptide Antibodies Against hCKIε and CKIα—To generate antibodies against hCKIε, the synthetic peptide MELRVGNKYRLGC, consisting of the first 12 amino-terminal amino acids of hCKIε (Fig. 1*B*) with a cysteine residue at the carboxyl terminus was coupled to *m*-maleimidobenzoyl-*n*-hydroxysuccinimide ester-activated bovine serum albumin (Harlow and Lane, 1988). To generate antibodies against hCKIα, the synthetic peptide CSGQGQQAQTPTGF, corresponding to the last 13 amino acid residues of hCKIα with an amino-terminal cysteine, was similarly coupled to *m*-maleimidobenzoyl-*n*-hydroxysuccinimide ester-activated keyhole limpet hemocyanin. Rabbits were injected with conjugated peptides at HRP, Inc., Denver, PA. Antiserum against the hCKIε peptide (expected to recognize both CKIδ and ε, see Fig. 2) is from rabbits UT31 and UT32, while rabbits UT3 and UT4 were immunized with the CKIα peptide-KLH conjugate.

Extracts from *E. coli* expressing hCKIε were subjected to 12% SDS-PAGE and transferred to a nitrocellulose filter in 12.5 mM Tris, pH 8.5, 86 mM glycine, 0.1% SDS, 20% methanol. After a 15-min fixation in 0.5% glutaraldehyde in phosphate-buffered saline, the filter was blocked with 3% nonfat dry milk and then incubated with a 1:1000 dilution of UT31 antiserum. Bound antibody was detected using a alkaline phosphatase-conjugated goat-antirabbit IgG (Bio-Rad) followed by incubation with bromochloroindolyl phosphate and nitro blue tetrazolium (Harlow and Lane, 1988).

Immunoprecipitation of Recombinant hCKIε—Recombinant hCKIε protein was immunoprecipitated from *E. coli* extracts (100 μg of protein) by the addition of 10 μl of rabbit antiserum UT31, followed 30 min later by 30 μl of a 50% slurry of protein A-agarose (Pierce). After an overnight incubation at 4 °C, the immunoprecipitates were washed three times in 50 mM Tris, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, once in the same buffer without SDS, and once in buffer B. Kinase reactions were performed as described above. To determine kinase autophosphorylation and phosphorylation of protein substrates, the reactions were stopped by boiling in sample buffer and analyzed on SDS-polyacrylamide gels. To determine peptide phosphorylation, protein A-agarose beads were removed by centrifugation, and acetic acid-treated supernatants were spotted onto P81 filters.

Mutagenesis of hCKIε and hCKIα2—To generate an inactive form of

[2] M. Hoekstra, personal communication.

hCKIε, lysine 38 was mutated to arginine (hCKIε^{K38R})(pDV35) by the unique site elimination method (Deng and Nickoloff, 1992) using the oligonucleotide C ACA CTC CAG CCG GAT GGC GAC TTC. Mutations in hCKIα2 were created using the pAlter-1 Altered Sites system (Promega). To facilitate subsequent cloning, an *Nco*I site (CCATGG) was created at the initiating ATG of hCKIα2(pELV3) using the oligonucleotide CT CTT CGT CTC TCA CCA TGG CGA GTA GC. hCKIα2^{K46A}(pELV6) contains an *Nco*I site at the initiating ATG and a mutation at codon 46 in which lysine was changed to alanine using the oligonucleotide GG AAG TGG CAG TGG CGC TAG AAT CTC AG. All mutations were confirmed by DNA sequencing.

Yeast Manipulation—*S. cerevisiae* strain hrr25Δ (*ura3–1, trp1–1, leu2–3, 112, his3–11, 15, can1–100, ade2–1, hrr25* Δ) was provided by Merl Hoekstra (Icos Corp.). Standard methods and media for yeast propagation and transformation were employed (Sherman *et al.*, 1986; Schiestl and Gietz, 1989).

Expression of Galactose-inducible CKI Expression Vectors—To obtain galactose-inducible expression of CKI in *S. cerevisiae*, we utilized a derivative of pRS305 (pRS305 2μ Gal1–10) that contains the 2μ origin of replication and the Gal1–10 promoter followed by a polylinker including *Nco*I and *Hin*dIII sites (provided by M. Hoekstra). hCKIε and hCKIε^{K38R} were excised from pBluescript SK(−) with *Nco*I and *Hin*dIII and inserted into the corresponding site of pRS305 2μ Gal1–10 (pDV49 and pKF119, respectively). hCKIα2 and hCKIα2^{K46A} were excised from pAlter by a *Hin*dIII/partial *Nco*I digest and similarly ligated into the expression vector (pKF120 and pKF121, respectively). As a positive control, a construct containing *HRR25*, likewise cloned into the *Nco*I site of pRS305 2μ Gal1–10, was obtained from M. Hoekstra. The expression plasmids were transformed into hrr25Δ cells and single transformants were selected and grown to saturation in synthetic complete media lacking leucine.

To ascertain whether the CKIα2 construct indeed produced active kinase, duplicate transformants of hCKIα2 and hCKIα2^{K46A} were grown in 10-ml synthetic medium with 2% raffinose as the carbon source to an A_{600} of 0.2. Kinase production was induced by the addition of galactose to a final concentration of 2%. After a 4-h induction at room temperature, the culture was harvested for soluble protein by glass bead disruption. Aliquots of extract containing 100 μg of protein were incubated with UT3 antiserum and protein A-agarose beads and the resulting immunoprecipitates used for a peptide kinase assay as described previously.

Figures—Polaroid photographs, autoradiographs and SDS-PAGE gels were scanned on a Scanmaker IIXE (MicroTek) at 300 dots per inch and assembled in Photoshop 2.5.1 (Adobe). Labels were applied in Canvas 3.5 (Deneba Software).

RESULTS

Isolation and Characterization of a Human Casein Kinase Iε cDNA Clone—A human placental cDNA library was screened using a 94-bp polymerase chain reaction product (*Probe 1*, Fig. 1*A*; see "Materials and Methods"). The two clones obtained appeared to be identical by restriction digest and partial sequence analysis. The cDNA insert in one of these clones, clone 10.1, was sequenced to completion. The clone 10.1 insert is 1.33 kilobase pairs long and includes 22 bp of 5′-untranslated sequence, an open reading frame of 1248 bp, and 55 bp of 3′-untranslated sequence including the stop codon (Fig. 1*B*). To see if additional related clones were present in the library, the 5′ portion of clone 10.1, from the 5′ *Eco*RI cloning site to the internal *Eco*RI site (*Probe 2*, Fig. 1*A*), was used as a probe to re-screen the library. Seven additional clones were isolated, three of which contained sequences related to, but distinct from the 10.1 clone. One of these related clones, clone 12.1, appears to be a partial but nearly full-length cDNA encoding human CKIδ. It contains an open reading frame identical to rat CKIδ for the first 380 amino acids, but lacks the first 20 bp (seven amino acids) of the CKIδ open reading frame. As discussed below, clones 10.1 and 12.1 appear to be derived from distinct genes.

The presumed start codon of the 1.33-kilobase pair clone 10.1 cDNA is at bases 23–25 (Fig. 1*B*). This ATG appears to be the authentic start site of the open reading frame because: 1) it lies within an optimal translational initiation consensus sequence of GCCATGG (Kozak, 1986, 1987); 2) it is immediately preceded by a stop codon in the same reading frame (bases 17–19); and 3) the predicted start site is consistent with those predicted for human and rat CKIδ as well as *S. cerevisiae* HRR25 (Hoekstra *et al.*, 1991; Graves *et al.*, 1993).[2] Conceptual translation of the open reading frame predicts a polypeptide of 416 amino acids and a molecular mass of 47,314 daltons, with a predicted pI of 10.5.

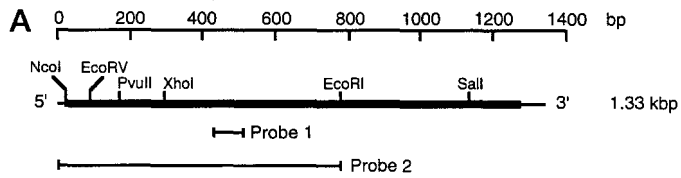
The clone 10.1 cDNA shows a high degree of sequence similarity to members of the CKI family at both the nucleotide and amino acid levels, with the greatest similarity to the CKIδ isoform. The predicted kinase domain (residues 9 to 293) is 53–98% identical and 71–99% similar to the kinase domains of other human and yeast members of the CKI family. Although isolated in a screen for CKIδ, this cDNA appears to be the product of a distinct gene and we therefore propose the name of human casein kinase I epsilon (hCKIε) for this new member of the casein kinase I gene family. Several lines of evidence indicate that hCKIε is not allelic with CKIδ. Over a stretch of 179 amino acids where hCKIε and hCKIδ are identical (Fig. 2), they share only 86% nucleic acid identity.[2, 3] While CKIε has 97.5% amino acid identity with hCKIδ over the kinase domain (residues 9–293, Fig. 2) it is only 53% identical in the carboxyl-terminal region (residues 294–416). Optimal alignment of the hCKIε and hCKIδ carboxyl-terminal domains requires two insertions in the hCKIε sequence. Overall, the hCKIε cDNA is only 77% identical to the hCKIδ cDNA open reading frame at the nucleic acid level and the 5′- and 3′-untranslated regions of the two cDNAs are completely divergent. The close similarity between the coding sequences of the two genes suggests a fairly recent gene duplication event.

Like other members of the CKI family, hCKIε is highly basic, with positively charged residues uniformly distributed over the length of the molecule. In common with other CKI family members, it lacks the salt-bridge forming APE motif seen in domain VIII of other protein kinases. The 123-residue carboxyl-terminal extension that follows the kinase domain begins with a 15-amino acid stretch (EDVDRERREHEREER) that contains 8 acidic and 6 basic residues. The homologous region of rat and human CKIδ contains 7 acidic and 7 basic residues in a 16-amino acid stretch. This motif is similar to the KEKE motif proposed to facilitate interactions with the intracellular multicatalytic protease (Realini *et al.*, 1994). The last 100 amino acids include 18 arginine and 11 proline residues and only 6 acidic residues.

Northern Blot Analysis of hCKIε Expression in Tissue Culture Cells—A Northern blot of poly(A)-selected RNA from log phase HeLa cells probed with hCKIε demonstrated the presence of a major message of 2.7 kilobases (kb) and a lower abundance message of 1.6 kb (Fig. 3*B*, *lanes 5* and *6*). These mRNAs are unique to hCKIε and not due to cross-hybridization with other known CKI isoforms, since in the same experiment a hCKIα2 probe identified mRNAs of 4.0 and 2.2 kb, and a hCKIδ probe identified species of 3.5, 2.0, and 1.8 kb (Fig. 3*B*, *lanes 1–4*). The hCKIε probe also identified species of 3.5 and 2.0 kb (Fig. 3*B*, *lane 6*); these may represent cross-hybridization with hCKIδ mRNAs.

A Northern blot was performed on total RNA extracted from eight cultured human cell lines to determine the spectrum of hCKIε expression. A major message of 2.9 kb was present in most cell lines, while a less abundant mRNA of 1.77 kb was also apparent (best seen in Fig. 3*A*, *lanes 9, 11,* and *12*, and also detected upon longer exposure in other cell lines and in poly(A)-selected RNA, data not shown). Of note, hCKIε mRNA was not detected in HL60 and MCF-7 cells (Fig. 3*A*, *lanes 7* and *10*).

[3] K. J. Fish and D. M. Virshup, unpublished results.



B

```
-22                                                                gaattccgggcaagagtgagcc -1

  1 ATG GAG CTA CGT GTG GGG AAC AAG TAC CGC CTG GGA CGG AAG ATC GGG AGC GGG TCC TTC  60
  1 M   E   L   R   V   G   N   K   Y   R   L   G   R   K   I   G   S   G   S   F   20

 61 GGA GAT ATC TAC CTG GGT GCC AAC ATC GCC TCT GGT GAG GAA GTC GCC ATC AAG CTG GAG 120
 21 G   D   I   Y   L   G   A   N   I   A   S   G   E   E   V   A   I   K   L   E   40

121 TGT GTG AAG ACA AAG CAC CCC CAG CTG CAC ATC GAG AGC AAG TTC TAC AAG ATG ATG CAG 180
 41 C   V   K   T   K   H   P   Q   L   H   I   E   S   K   F   Y   K   M   M   Q   60

181 GGT GGC GTG GGG ATC CCG TCC ATC AAG TGG TGC GGA GCT GAG GGC GAC TAC AAC GTG ATG 240
 61 G   G   V   G   I   P   S   I   K   W   C   G   A   E   G   D   Y   N   V   M   80

241 GTC ATG GAG CTG CTG GGG CCT AGC CTC GAG GAC CTG TTC AAC TTC TGT TCC CGC AAA TTC 300
 81 V   M   E   L   L   G   P   S   L   E   D   L   F   N   F   C   S   R   K   F  100

301 AGC CTC AAG ACG GTG CTG CTC TTG GCC GAC CAG ATG ATC AGC CGC ATC GAG TAT ATC CAC 360
101 S   L   K   T   V   L   L   L   A   D   Q   M   I   S   R   I   E   Y   I   H  120

361 TCC AAG AAC TTC ATC CAC CGG GAC GTC AAG CCC GAC AAC TTC CTC ATG GGG CTG GGG AAG 420
121 S   K   N   F   I   H   R   D   V   K   P   D   N   F   L   M   G   L   G   K  140

421 AAG GGC AAC CTG GTC TAC ATC ATC GAC TTC GGC CTG GCC AAG AAG TAC CGG GAC GCC CGC 480
141 K   G   N   L   V   Y   I   I   D   F   G   L   A   K   K   Y   R   D   A   R  160

481 ACC CAC CAG CAC ATT CCC TAC CGG GAA AAC AAG AAC CTG ACC GGC ACG GCC CGC TAC GCT 540
161 T   H   Q   H   I   P   Y   R   E   N   K   N   L   T   G   T   A   R   Y   A  180

541 TCC ATC AAC ACG CAC CTG GGC ATT GAG CAA AGC CGT CGA GAT GAC CTG GAG AGC CTG GGC 600
181 S   I   N   T   H   L   G   I   E   Q   S   R   R   D   D   L   E   S   L   G  200

601 TAC GTG CTC ATG TAC TTC AAC CTG GGC TCC CTG CCC TGG CAG GGG CTC AAA GCA GCC ACC 660
201 Y   V   L   M   Y   F   N   L   G   S   L   P   W   Q   G   L   K   A   A   T  220

661 AAG CGC CAG AAG TAT GAA CGG ATC AGC GAG AAG AAG ATG TCA ACG CCC ATC GAG GTC CTC 720
221 K   R   Q   K   Y   E   R   I   S   E   K   K   M   S   T   P   I   E   V   L  240

721 TGC AAA GGC TAT CCC TCC GAA TTC TCA ACA TAC CTC AAC TTC TGC CGC TCC CTG CGG TTT 780
241 C   K   G   Y   P   S   E   F   S   T   Y   L   N   F   C   R   S   L   R   F  260

781 GAC GAC AAG CCC GAC TAC TCT TAC CTA CGT CAG CTC TTC CGC AAC CTC TTC CAC CGG CAG 840
261 D   D   K   P   D   Y   S   Y   L   R   Q   L   F   R   N   L   F   H   R   Q  280

841 GGC TTC TCC TAT GAC TAC GTC TTT GAC TGG AAC ATG CTG AAA TTC GGT GCA GCC CGG AAT 900
281 G   F   S   Y   D   Y   V   F   D   W   N   M   L   K   F   G   A   A   R   N  300

901 CCC GAG GAT GTG GAC CGG GAG CGG CGA GAA CAC GAA CGC GAG GAG AGG ATG GGG CAG CTA 960
301 P   E   D   V   D   R   E   R   R   E   H   E   R   E   E   R   M   G   Q   L  320

961 CGG GGG TCC GCG ACC CGA GCC CTG CCC CCT GGC CCA CCC ACG GGG GCC ACT GCC AAC CGG 1020
321 R   G   S   A   T   R   A   L   P   P   G   P   P   T   G   A   T   A   N   R  340

1021 CTC CGC AGT GCC GCC GAG CCC GTG GCT TCC ACG CCA GCC TCC CGC ATC CAG CCG GCT GGC 1080
 341 L   R   S   A   A   E   P   V   A   S   T   P   A   S   R   I   Q   P   A   G  360

1081 AAT ACT TCT CCC AGA GCG ATC TCG CGG GTC GAC CGG GAG AGG AAG GTG AGT ATG AGG CTG 1140
 361 N   T   S   P   R   A   I   S   R   V   D   R   E   R   K   V   S   M   R   L  380

 141 CAC AGG GGT GCG CCC GCC AAC GTC TCC TCC TCA GAC CTC ACT GGG CGG CAA GAG GTC TCC 1200
 381 H   R   G   A   P   A   N   V   S   S   S   D   L   T   G   R   Q   E   V   S  400

1201 CGG ATC CCA GCC TCA CAG ACA AGT GTG CCA TTT GAC CAT CTC GGG AAG TGA ggagagccccca 1263
 401 R   I   P   A   S   Q   T   S   V   P   F   D   H   L   G   K   *                417

1264 ttggaccagtgtttgcttagtgtcttcactgtattttctggaattc                                  1309
```

FIG. 1.

```
          1                   *                                      50
hCKIε              ME LRVGNKYRLG RKIGSGSFGD IYLGANIASG EEVAIKLECV
hCKIδ              -- -----R---- ---------- ----TD--A- ----------
hCKIα     MASSSGSKA- FI--G--K-V ---------- ---AI--TN- ----V---SQ
HRR25              -D ----R-F-I- ---------- --H-T-LI-- --------SI

          51                                                     100
hCKIε     KTKHPQLHIE SKFYKMMQGG VGIPSIKWCG AEGDYNVMVM ELLGPSLEDL
hCKIδ     ---------- --I------- ----T-R--- ---------- ----------
hCKIα     -AR----LY- --L--IL--- ----H-R-Y- Q-K----L-- D---------
HRR25     RSR----DY- -RV-RYLS-- ----F-R-F- R--E--A--I D---------

          101                                                    150
hCKIε     FNFCSRKFSL KTVLLLADQM ISRIEYIHSK NFIHRDVKPD NFLMGLGKKG
hCKIδ     ---------- ---------- ---------- ---------- ----------
hCKIα     ------R-TM ----M----- ------V-T- ------I--- -----I-RHC
HRR25     --Y-H-R--F ---IM--L-- FC--Q---GR S-----I--- -----V-RR-

          151                                                    200
hCKIε     NLVYIIDFGL AKKYRDARTH QHIPYRENKN LTGTARYASI NTHLGIEQSR
hCKIδ     ---------- ---------- ---------- ---------- ----------
hCKIα     -KLFL----- ------N--R -------D-- ---------- -A--------
HRR25     ST-HV----- S-----FN-- R--------S ---------V ----------

          201                          ## ##                     250
hCKIε     RDDLESLGYV LMYFNLGSLP WQGLKAATKR QKYERISEKK MSTPIEVLCK
hCKIδ     ---------- ---------- ---------- ---------- ----------
hCKIα     ---M------ -----RT--- ---------K ----K----- ----V-----
HRR25     ---------- -I--CK---- ------T--K ---D--M--- LNVSV-T--S

          251                                                    300
hCKIε     GYPSEFSTYL NFCRSLRFDD KPDYSYLRQL FRNLFHRQGF SYDYVFDWNM
hCKIδ     ------A--- ---------- ---------- ---------- ----------
hCKIα     -F-A--AM-- -Y--G---EE A---M----- --I--RTLNH Q---T---T-
HRR25     -L-L--QEYM AY-KN-K--E ----LF-AR- -KD-SIKLEY HN-HL---T-

          *                                                      350
hCKIε     LKFGAARNPE DVDRERREHE REERMGQLRG SATRALPPGP PTGATANRLR
hCKIδ     -----S-AAD DAE----..D ----LRHS-N P---GLP... ..STASG---
hCKIα     --QK--QQAA SSSGQGQQAQ TPTGKQTDKT KSNMKG
HRR25     -RYTK-MVEK QR-LLIEKGD LNANSNAASA SNSTDNKSET FNKIKLLAMK

          351                                                    400
hCKIε     SAAEPVASTP ASRIQPAGNT SPRAISRVDR ERKVSMRLHR GAPANVSSSD
hCKIδ     GTQEVAPP-- LTPTSHTAN- ---PV-GME- ---------- ---V-I----
HRR25     KFPTHFHYYK NEDKHNPSPE EIKQQTILNN NAAS-LPEEL LNALDKGME.

          401
hCKIε     LTGRQEVSRI PASQTSVPFD HLGK
hCKIδ     -----DT--M ST--IPGRVA SS-LQSVVHR
HRR25     NLRQ-QPQQQ VQSSQPQPQP QQLQQQPNGQ......
```

FIG. 2. **Alignment of selected casein kinase I family members.** Alignment of hCKIε, hCKIδ, hCKIα2, and HRR25, generated by the PILEUP program of the GCG package (Devereux *et al.*, 1984). Amino acid identities are indicated with a hyphen (–), a *star* marks the first and last residues of the kinase domain, deletions are indicated by a *dot*, and # marks the putative nuclear localization signal. HRR25 is truncated at residue 421. The sequences used to generate antisera are *underlined*.

This broad distribution of hCKIε expression is similar to that seen with rat and rabbit CKIδ, which was detected in virtually all tissues examined (Graves *et al.*, 1993).

Genomic Localization of hCKIα, hCKIδ, and hCKIε—Mutations in human genes involved in DNA metabolism may have diverse clinical manifestations. It was of interest to determine whether: 1) hCKIε and hCKIδ mapped to distinct loci, and 2) any of the known CKI genes were linked to any mapped human genetic disorders.

The genomic location of the CKI-related genomic clones A115C12, D76C5, E14G10, and cos3.3 (see "Materials and Methods") was determined by fluorescent *in situ* hybridization to metaphase chromosomes (Fig. 4). Hybridization efficiency for each genomic clone was greater than 90% for both metaphase and interphase chromosomes. A115C12 (hCKIα) hybridized to a D group chromosome (chromosomes 13–15), D76C5 (hCKIδ) hybridized to an E group chromosome (chromosomes 16–18), and E14G10 and cos3.3 (both hCKIε) hybridized to a G group chromosome (chromosomes 21, 22). Because E14G10 also hybridized to the Y chromosome, further localization of hCKIε was performed only with cos3.3.

Chromosomal assignments were made by co-hybridizing a biotin-labeled CKI-containing genomic clone with a uniquely haptenated fiduciary marker originating from a candidate chromosome. Coincident hybridizations were achieved when fiduciary markers for chromosome 13 (D13S6), chromosome 17

FIG. 1. **Map and sequence of human CKIε.** *A*, map of clone 10.1 encoding hCKIε. Clone 10.1 is depicted schematically with the deduced protein coding sequence shown as a *box* and the 5′- and 3′-untranslated regions shown as a single line. Probes 1 and 2 are defined under "Materials and Methods." *B*, nucleotide and predicted amino acid sequence of human cDNA encoding hCKIε. Amino acids are indicated in single-letter code. The stop codon is indicated by a *star*. The *Eco*RI linkers on either end of the library cDNA are in *italics*.

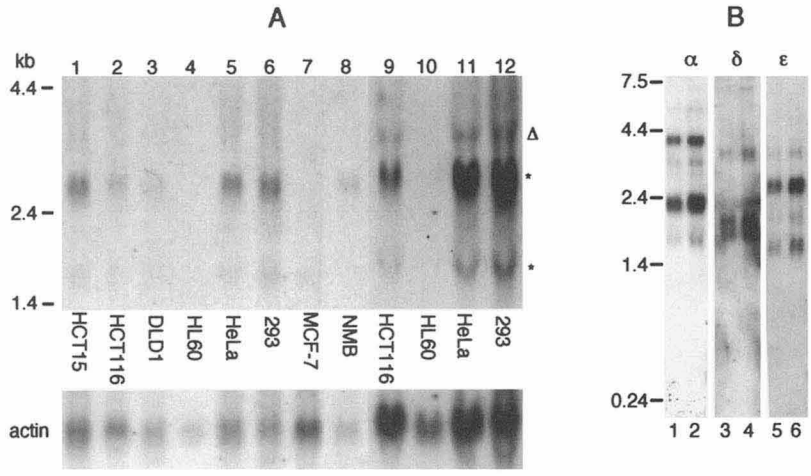


FIG. 3. **Northern blot analysis of hCKIε expression.** *A*, hCKIε is expressed in diverse tissue culture cell lines. *Lanes 1–8* each contain 16 μg and *lanes 9–12* each contain 50 μg of total cellular RNA isolated from the indicated human cell lines. In the *upper panel*, the filter was probed with ^{32}P-labeled full-length hCKIε cDNA, while in the *lower panel* the same filter was probed with ^{32}P-labeled mouse α actin cDNA. A *star* marks the 2.7- and 1.6-kb hCKIε mRNAs, while the *triangle* (Δ) indicates a 3.5-kb mRNA species that may represent cross-reactivity with hCKIδ mRNA. *HCT 15,* colon adenocarcinoma; *HCT 116*, colon carcinoma; *DLD 1*, colon carcinoma; *HL60,* promyelocytic cell line; *HeLa*, cervical carcinoma; *293*, adenovirus-transformed embryonal kidney; *MCF7*, breast adenocarcinoma; *NMB*, neuroblastoma. *B*, hCKIε mRNA is distinct from the mRNA of other members of the human CKI family. A Northern blot containing poly(A) selected RNA from log phase HeLa cells was probed with ^{32}P-labeled hCKIα2 (*lanes 1* and *2*), hCKIδ (*lanes 3* and *4*), or hCKIε (*lanes 5* and *6*). *Lanes 1, 3*, and *5* each contain 2 μg, and *lanes 2, 4*, and *6* each contain 4 μg of RNA.

(Yurov *et al.*, 1987; Alexandrov *et al.*, 1988), and chromosome 22 (D22S9) were co-hybridized with A115C12, D76C5, and cos3.3, respectively (data not shown).

Chromosomal localizations were refined by determining the chromosomal address in terms of FLpter%. As described by Lichter *et al.* (1990), the distance the hybridization signal was from the pterminus (pter) was expressed as a percentage of the actual contour length of the chromosome (pter to qter) and denoted as FLpter%. The results for each genomic clone are summarized in Fig. 4. A115C12 mapped to chromosome 13 with a FLpter% value of 39.7 ± 6.4%. D76C5 mapped to chromosome 17 with a FLpter% of 93.8 ± 4.8%. cos3.3 mapped to chromosome 22 with a FLpter% value of 73.7 ± 4.4%. When compared to banded chromosomes A115C12 originates from chromosome 13q13.1–14.1, confirming the recent report of Tapia and co-workers (Tapia *et al.*, 1994). The fact that only a single genomic locus was found for hCKIα supports the hypothesis that hCKIα, hCKIα2, and hCKIαL (also called CKIα3) are splice variants rather than the products of unique genes. D76C5 localizes to chromosome 17q25.2–25.3 in a region containing the thymidine kinase gene. The gene encoding hCKIε is located on chromosome 22q12.3–13.1 telomeric to the NF2/MERLIN locus and lies in a region frequently deleted in familial and sporadic meningiomas (OMIM, 1994).

Expression of Recombinant Casein Kinase Iε in E. coli—The high degree of similarity between hCKIε and hCKIδ strongly suggests that hCKIε encodes an active kinase. To confirm this, and as an initial step in characterizing hCKIε activity, the hCKIε coding sequence was cloned into the pET16b bacterial expression vector ("Materials and Methods"). Cultures of *E. coli* strain BL21(DE3) pLysS transformed with empty pET16b vector or vector containing hCKIε were grown as described under "Materials and Methods." After induction with isopropyl-1-thio-β-D-galactoside, cells carrying the hCKIε construct contained a 48-kDa protein that reacted with an anti-CKIδ/ε antibody on immunoblot (Fig. 5*A*). As seen with CKIδ (Graves *et al.*, 1993), expression of hCKIε was at relatively low levels, since no significant bands of this size were evident on Coomassie-stained gels of total cellular extract (data not shown).

Phosphorylation of Peptide by Crude Extracts—Casein kinase I family members are characterized by their ability to phosphorylate substrates with acidic or phosphoserine residues amino-terminal to the target serine or threonine. To determine if overexpressed hCKIε had a similar sequence specificity, the D4 peptide (see "Materials and Methods") was incubated with soluble extracts from *E. coli* carrying the hCKIε construct. CKI activity was present at low levels before induction and increased 5.5-fold after induction (Fig. 5*B*). Lower levels of D4 phosphorylation were found using extracts from induced cells carrying empty expression vector. These data indicate that hCKIε can phosphorylate a well-defined casein kinase I substrate.

Phosphorylation of Protein Substrates by hCKIε Immunoprecipitates—The activity of hCKIε on a variety of protein substrates was tested, taking advantage of the finding that hCKIε was active after immunoprecipitation from *E. coli* lysates with anti-hCKIε antiserum. As expected for members of the casein kinase I family, immunoprecipitates from induced cells phosphorylated phosvitin (Fig. 5*C*, *lane 1*) and casein (*lane 2*), but not histone H1 (*lane 3*). Inclusion of the antigenic peptide in the immunoprecipitation reaction significantly reduced the kinase activity in the pellet (*lane 5*). Immunoprecipitated hCKIε actively autophosphorylated (Fig. 5*C*, *arrow*), an activity well described for other CKI family members.

Inhibition of hCKIε by the Casein Kinase I Inhibitor CKI-7—The compound *N*-(2-aminoethyl)-5-chloro-isoquinoline-8-sulfonamide (CKI-7) has been reported to specifically inhibit most (Chijiwa *et al.*, 1989), although not all (Vancura *et al.*, 1993) isoforms of casein kinase I. To determine if CKI-7 (obtained from Seikagaku Corp.) inhibits hCKIε activity, increasing concentrations of the compound were incubated with immunoprecipitated hCKIε and the D4 peptide. CKI-7 inhibited phosphorylation with half-maximal inhibition of kinase activity at 18 μM CKI-7 (Fig. 5*D*). These results are consistent with those of Chijiwa *et al.* (1989), who found an K_i of 8.5 μM using rabbit skeletal muscle CKI, and Graves *et al.* (1993), who described an IC_{50} of 12 μM using recombinant rat CKIδ. Taken together these results support the hypothesis that hCKIε is a bona fide member of the casein kinase I gene family.

Rescue of the hrr25Δ Slow Growth Phenotype by hCKIε but Not hCKIα2—Sequence analysis of the casein kinase I gene family members suggests that hCKIε and hCKIδ are most closely related to *S. cerevisiae HRR25* and *S. pombe* hhp1$^+$ and

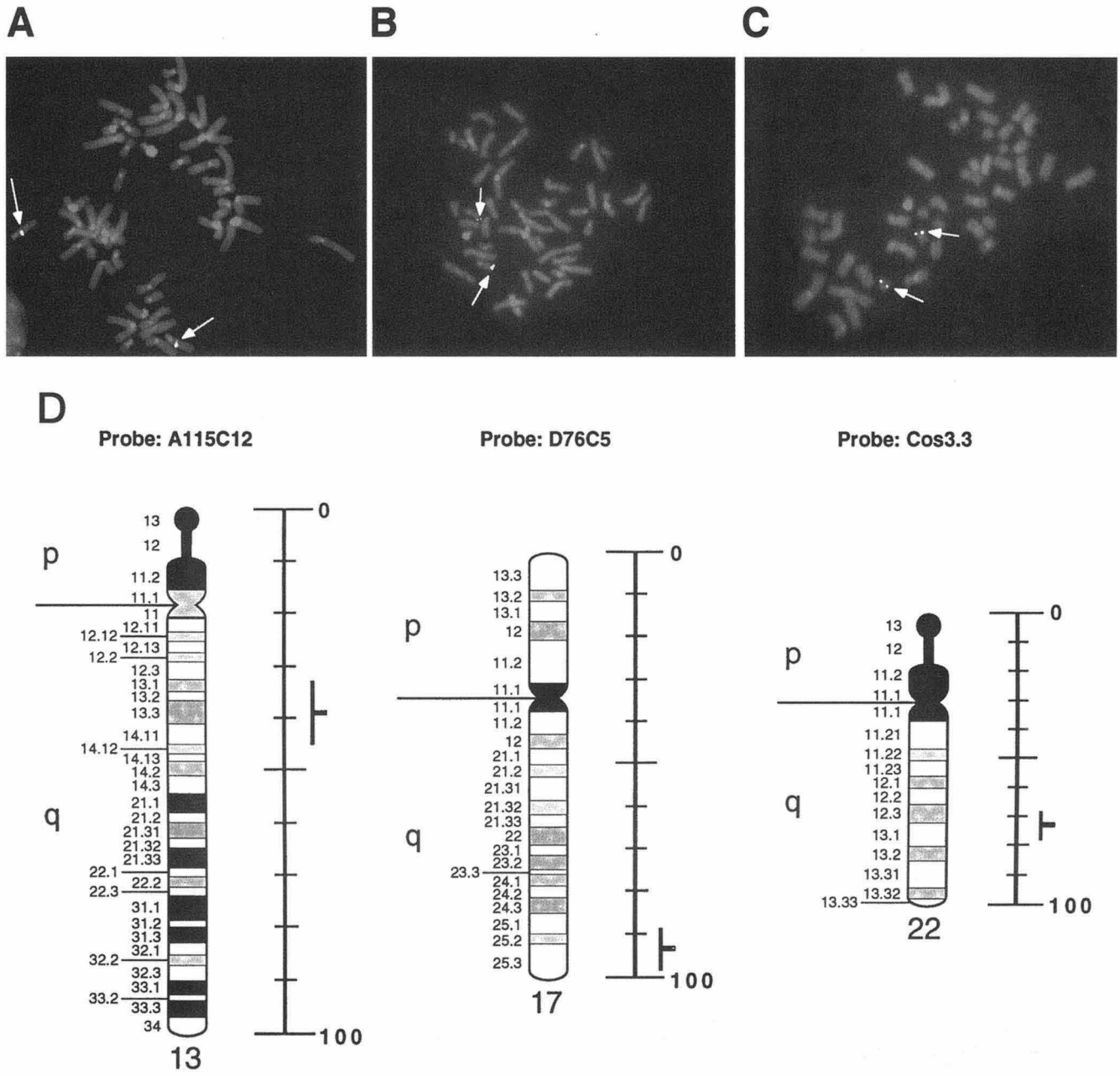


FIG. 4. **Fluorescent *in situ* hybridization of CKI-containing genomic clones to human metaphase chromosomes.** Hybridized probes were detected with Cy3-avidin. *A*, hybridization of biotinylated A115C12 (hCKIα) to a D group chromosome. *B*, hybridization of biotinylated D76C5 (hCKIδ) to an E group chromosome. *C*, hybridization of biotinylated cos3.3 (hCKIε) to a G group chromosome. *D*, refined localization of CKI-containing genomic clones using FLpter%. The *left, middle,* and *right panels* show the FLpter% map positions (within 2 standard deviations) alongside chromosome ideograms (Francke, 1994) of chromosome 13 for A115C12 (39.7 ± 6.4%), chromosome 17 for D76C5 (93.8 ± 4.8%), and chromosome 22 for cos3.3 (73.7 ± 4.4%). Ideograms represent chromosome preparations at the 850 band level.

hhp2$^+$, the kinases involved in DNA repair. A prominent phenotype of yeast with a deletion of the HRR25 gene is very slow growth with a doubling time of greater than 12 h. To determine whether human CKI isoforms could complement this growth defect, cDNAs encoding hCKIε and hCKIα2 were cloned into a galactose-inducible high copy expression vector (see "Materials and Methods"). Inducible expression of the cloned proteins in hrr25Δ cells was confirmed by immunoblot analysis of extracts from induced cultures (data not shown). We ascertained that active hCKIα2 was produced by incubating extracts from uninduced and induced cells with CKIα antiserum and assaying the immunoprecipitates for CKI activity.

As shown by the dilution spot assay (Fig. 6), under inducing conditions hCKIε rescued the slow growth phenotype of the hrr25Δ cells almost as well as HRR25. The kinase activity of hCKIε was required to rescue, since a kinase-deficient mutant did not complement. Hoekstra and co-workers have found that human CKIδ also complements the hrr25Δ cells,[2] consistent with the high degree of homology of the two proteins. Interestingly, the construct expressing hCKIα2 grew almost as poorly as the deletion mutant bearing vector alone. Thus, hCKIα2 and hCKIε, while closely related, are functionally distinct.

DISCUSSION

Casein Kinase I Epsilon Is a New Member of the Casein Kinase I Gene Family—This report details the isolation and cDNA cloning of a new member of the casein kinase I gene family. This kinase, hCKIε, contains a kinase domain highly related to other members of the family, and a carboxyl-terminal extension most closely related to that of CKIδ (Graves *et al.*,

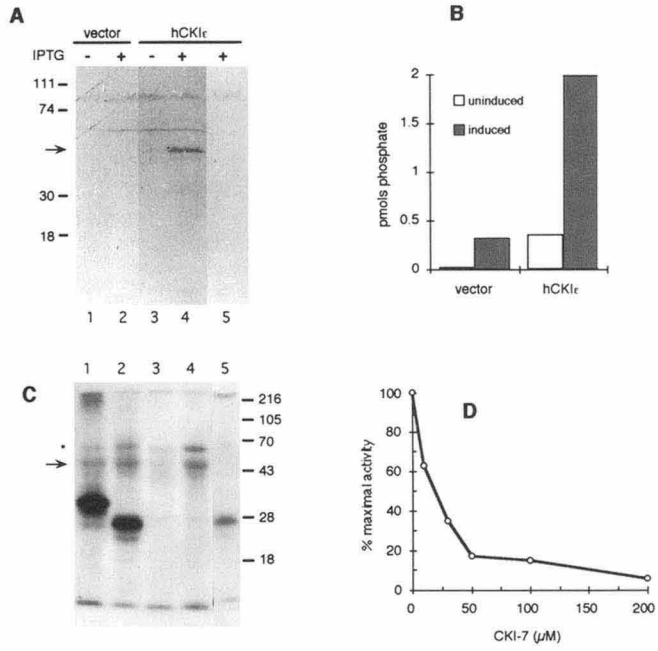


FIG. 5. **Expression of active hCKIε in *E. coli*.** *A*, hCKIε encodes a 48-kDa polypeptide *E. coli* strain BL21(DE3) pLysS containing expression vector pET16b with or without hCKIε cDNA insert were lysed before or after a 2-h induction at 37 °C with 0.4 mM isopropyl-1-thio-β-D-galactoside (*IPTG*). Expression of hCKIε was detected by Western blot with a 1:1000 dilution of UT31 antiserum in the absence (*lanes 1–4*) or presence (*lane 5*) of 1 μg of the antigenic peptide. *B*, D4 peptide phosphorylation in crude extracts. Soluble extracts were prepared from isopropyl-1-thio-β-D-galactoside-induced and uninduced *E. coli* carrying pET16b or pET16b-hCKIε. Kinase activity on the peptide substrate DDDDVASLPGLRRR was quantitated as described ("Materials and Methods"). *C*, substrate specificity of hCKIε. Recombinant hCKIε was immunoprecipitated from extracts with UT31 antiserum and incubated in the presence of [γ-^{32}P]ATP with 1 μg of phosvitin (*lane 1*), 10 μg of casein (*lanes 2* and *5*), 10 μg of histone H1 (*lane 3*), or no added substrate (*lane 4*). The kinase immunoprecipitation in *lane 5* was performed in the presence of excess antigenic peptide. The *arrow* indicates the position of autophosphorylated hCKIε (48 kDa). The 60-kDa band (marked with *) is a co-precipitating *E. coli* protein, since a band of identical size co-precipitates with a truncated 44-kDa hCKIε (data not shown). *D*, hCKIε kinase activity is inhibited by CKI-7. The activity of immunoprecipitated hCKIε on the substrate peptide D4 was determined at increasing concentrations of the CKI inhibitor CKI-7.

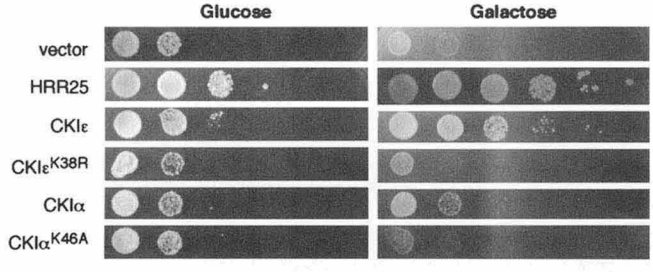


FIG. 6. **Rescue of *S. cerevisiae* hrr25Δ slow growth phenotype by hCKIε but not hCKIα2.** *S. cerevisiae* with a deletion of *HRR25* were transformed with a high copy plasmid bearing the indicated cDNAs under the control of the Gal1–10 operator. Serial 10-fold dilutions of saturated cultures were spotted on selective medium containing glucose or galactose as the sole carbon source and incubated for 4 days at 30 °C.

1993). Nucleic acid sequence and chromosomal localization indicate that hCKIε is the product of a distinct gene, rather than an allele of CKIδ.

Many of the Casein Kinase I Genes Appear to be Redundant—A prominent feature of the casein kinase I gene family tree is the redundancy present in several of its branches. In fission yeast at least two pairs of CKI isoforms have been found. One pair, hhp1$^+$ and hhp2$^+$, play overlapping roles in DNA repair (Dhillon and Hoekstra, 1994). A second pair, cki1$^+$ and cki2$^+$, while homologous in the kinase domain, are distinct in the carboxyl-terminal tail and appear to play distinct roles *in vivo* (Wang *et al.*, 1992, 1994). In budding yeast, YCK1 and YCK2 form an essential gene pair (Robinson *et al.*, 1992, 1993), while HRR25 does not appear to have a partner. In mammals, CKIα and CKIβ are most closely related, both containing only a short (24–35 amino acid) carboxyl-terminal extension. It is of note, however, that CKIβ has not yet been isolated from human cDNA libraries. Using bovine CKIγ as a probe, we have isolated several distinct human CKIγ cDNA clones, suggesting multiple related genes in this branch of the family (data not shown). The identification of hCKIε, with its high degree of homology with CKIδ, demonstrates that this branch of the CKI tree has redundancy as well.

The sequence similarity of various CKI isoforms suggests they may fulfill similar but not necessarily the same functions *in vivo*. Thus, CKIε and CKIδ may serve overlapping rather than identical functions in mammals. A functional overlap (as opposed to complete redundancy) may result from subtle or overt differences in enzyme regulation, substrate specificity, intracellular localization, or tissue specific expression. Thus, the redundancy suggested by the high degree of sequence similarity does not rule out clinical manifestations caused by mutations or disruptions of a single gene.

Several lines of evidence suggest that hCKIε may play a role in DNA metabolism. First, the related CKIα can inhibit the replication function of large T antigen in SV40 DNA replication (Cegielska and Virshup, 1993; Cegielska *et al.*, 1994). Importantly, hCKIε and hCKIδ but not hCKIα2 rescues the growth phenotype of hrr25Δ, a yeast kinase involved in DNA repair. This appears to confirm the suggestion of Kuret and co-workers (Kearney *et al.*, 1994) who found that the CKIδ/ε branch of the mammalian CKI family are most similar to HRR25, Hhp1 and Hhp2, the yeast kinases associated with DNA repair defects. Of note in this regard, hCKIε shares with hCKIα, hCKIδ, HRR25, Hhp1, and Hhp2 a potential nuclear localization signal (KRQK, residues 221–224) and purification of CKI activity from mammalian nuclei has yielded forms of CKI similar in size (50–55 kDa) to hCKIε (Tuazon and Traugh, 1991). It was therefore of interest to determine whether any of the CKI genes were linked to any known DNA repair disorders. The hCKIε gene indeed resides in a region (22q12.3–13.1) implicated in familial and sporadic meningiomas distinct from mutations in NF2 or the c-*sis* proto-oncogene (OMIM, 1994). Further studies will be required to determine if the hCKIε gene is indeed mutant in this or other diseases.

Function of Carboxyl-terminal Extension—A prominent feature of hCKIε is its carboxyl-terminal extension, a domain it shares with CKIδ. Three distinct functions of the carboxyl-terminal extension of other casein kinase I family members have been described to date. 1) Regulation of over-expressed rat CKIδ by heparin was abolished by removal of the tail (Graves *et al.*, 1993). 2) Activity of *S. pombe* Cki1, using casein as a substrate, was increased severalfold by removal of the tail (Carmel *et al.*, 1994). 3) Removal of the last 80 amino acids of YCK2, including a carboxyl-terminal prenylation signal, significantly impaired its ability to rescue a *yck1/yck2* double mutant (Robinson *et al.*, 1993). Thus, sequences in the carboxyl terminus can be involved in enzyme regulation, enzyme activity, and membrane localization. The ability of hCKIε (with a 123-amino acid carboxyl-terminal extension) but not hCKIα2 (with a 35-amino acid carboxyl-terminal extension) to rescue the growth defect of hrr25Δ yeast also suggests an important biological role for the carboxyl-terminal domain. How this do-

main functions in mammalian forms of CKI to regulate its *in vivo* function remains to be determined.

Acknowledgments—We thank Erica Vielhaber for mutagenesis of hCKIα, Mark Curran, Dick Lemons, Matt Rebentisch, and Darryl Gietzen for technical help, Andrew Thorburn for reviewing the manuscript, Joie Rowles and Melanie Cobb for bovine CKI clones, and Merl Hoekstra for shared reagents and productive discussions. Screening of the P1 library was facilitated by Mark Leppert and the Technical Access Section of the Utah Center for Human Genome Research (5P30 HG00199).

REFERENCES

Alexandrov, I. A., Mitkevich, S. P., and Yurov, Y. B. (1988) *Chromosoma* **96,** 443–453

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994) *Current Protocols in Molecular Biology,* Wiley-Interscience, New York

Brockman, J. L., Gross, S. D., Sussman, M. R., and Anderson, R. A. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 9454–9458

Carmel, G., Leichus, B., Cheng, X., Patterson, S. D., Mirza, U., Chait, B. T., and Kuret, J. (1994) *J. Biol. Chem.* **269,** 7304–7309

Casnellie, J. E. (1991) *Methods Enzymol.* **200,** 115–120

Cegielska, A., and Virshup, D. M. (1993) *Mol. Cell. Biol.* **13,** 1202–1211

Cegielska, A., Moarefi, I., Fanning, E., and Virshup, D. M. (1994) *J. Virol.* **68,** 269–275

Chijiwa, T., Hagiwara, M., and Hidaka, H. (1989) *J. Biol. Chem.* **264,** 4924–4927

Curran, M. E., Landes, G. M., and Keating, M. (1992) *Genomics* **12,** 729–737

DeMaggio, A. J., Lindberg, R. A., Hunter, T., and Hoekstra, M. F. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 7008–7012

Deng, W. P., and Nickoloff, J. A. (1992) *Anal. Biochem.* **200,** 81–88

Devereux, J., Haeberli, P., and Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–395

Dhillon, N., and Hoekstra, M. F. (1994) *EMBO J.* **13,** 2777–2788

Flotow, H., and Roach, P. J. (1989) *J. Biol. Chem.* **264,** 9126–9128

Flotow, H., and Roach, P. J. (1991) *J. Biol. Chem.* **266,** 3724–3727

Flotow, H., Graves, P. R., Wang, A., Fiol, C. J., Roeske, R. W., and Roach, P. J. (1990) *J. Biol. Chem.* **265,** 14264–14269

Francke, U. (1994) *Cytogen. Cell Genet.* **65,** 206–219

Graves, P. R., Haas, D. W., Hagedorn, C. H., DePaoli-Roach, A. A., and Roach, P. J. (1993) *J. Biol. Chem.* **268,** 6394–6401

Harlow, E., and Lane, D. (1988) *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Hoekstra, M. F., Liskay, R. M., Ou, A. C., DeMaggio, A. J., Burbee, D. G., and Heffron, F. (1991) *Science* **253,** 1031–1034

Hoekstra, M. F., Dhillon, N., Carmel, G., DeMaggio, A. J., Lindberg, R. A., Hunter, T., and Kuret, J. (1994) *Mol. Biol. Cell* **5,** 877–886

Kearney, P. H., Ebert, M., and Kuret, J. (1994) *Biochem. Biophys. Res. Commun.* **203,** 231–236

Kozak, M. (1986) *Cell* **44,** 283–292

Kozak, M. (1987) *J. Mol. Biol.* **196,** 947–950

Landes, G. M., Curran, M. E., and Keating, M. T. (1995) *Cytogen. Cell Genet.* in press

Lichter, P., Cremer, T., Borden, J., Manulelidis, L., and Ward, D. C. (1988) *Hum. Genet.* **80,** 224–234

Lichter, P., Tang, C. J., Call, K., Hermanson, G., Evans, G. A., Houseman, D., and Ward, D. (1990) *Science* **247,** 64–69

OMIM (1994) *Meningioma, MIM# 156100,* November 6, 1994, Johns Hopkins University, Baltimore, MD

Realini, C., Rogers, S. W., and Rechsteiner, M. (1994) *FEBS Lett.* **348,** 109–113

Robinson, L. C., Hubbard, E. J., Graves, P. R., DePaoli, R. A. A., Roach, P. J., Kung, C., Haas, D. W., Hagedorn, C. H., Goebl, M., and Culbertson, M. R. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 28–32

Robinson, L. C., Menold, M. M., Garrett, S., and Culbertson, M. R. (1993) *Mol. Cell. Biol.* **13,** 2870–2881

Rowles, J., Slaughter, C., Moomaw, C., Hsu, J., and Cobb, M. H. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 9548–9552

Schiestl, R. H., and Gietz, R. D. (1989) *Curr. Genetics* **16,** 339–346

Shepherd, N. S., Pfrogner, B. D., Coulby, J. N., Ackerman, S. L., Vaidyanathan, G., Sauer, R. H., Balkenhol, T. C., and Steinberg, N. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 2629–2633

Sherman, F., Fink, G. R., and Hicks, J. B. (1986) *Methods in Yeast Genetics,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Studier, F. W. (1991) *J. Mol. Biol.* **219,** 37–44

Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) *Methods Enzymol.* **185,** 60–89

Tapia, C., Featherstone, T., Gómez, C., Taillon-Miller, P., Allende, C. C., and Allende, J. E. (1994) *FEBS Lett.* **349,** 307–312

Tuazon, P. T., and Traugh, J. A. (1991) *Adv. Second Messenger Phosphoprotein Res.* **23,** 123–164

Vancura, A., O'Connor, A., Patterson, S. D., Mirza, U., Chait, B. T., and Kuret, J. (1993) *Arch. Biochem. Biophys.* **305,** 47–53

Wang, P. C., Vancura, A., Mitcheson, T. G., and Kuret, J. (1992) *Mol. Biol. Cell* **3,** 275–86

Wang, P. C., Vancura, A., Desai, A., Carmel, G., and Kuret, J. (1994) *J. Biol. Chem.* **269,** 12014–12023

Yurov, Y. B., Mitkevich, S. P., and Alexandrov, I. A. (1987) *Hum. Genet.* **76,** 157–164