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Identification of Murine cdk10: Association With Ets2 Transcription Factor and Effects on the Cell Cycle

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Abstract Cyclin-dependent kinases (cdks) are the catalytic subunits of a large family of serine/threonine protein kinases whose best-characterized members are key regulators of eukaryotic cell cycle progression. They are activated by binding to regulatory subunits generally termed as cyclins. Cdk10 is a cdc2-related kinase that contains the canonical regulatory Tyr and Thr residues present in all protein kinases and a PSTAIRE-like motif named PISSLRE. Although little is known about this protein, human cdk10 has been shown to encode two different isoforms, each having a distinct function. They differ at both the carboxy- and amino-terminals, although most of the amino acid sequence is predicted to be identical for the two isoforms. A role at the G2/M transition has been suggested for an isoform of cdk10, while the alternative splicing form interacts with the N-terminus of the Ets2 transcription factor. Here we report the cloning and the functional characterization of a cDNA encoding the murine homologue of cdk10. Unlike its human counterpart, only one murine cdk10 protein has been identified, and this unique murine cdk10 cDNA encodes a putative protein of 360 amino acids. Comparison of the amino acid sequences of murine and human cdk10 shows high homology. Murine cdk10 binds Ets2 transcription factors in vitro, does not show a direct involvement in the G2/M transition and, therefore, does not affect the proliferation rate of the cell lines analyzed. J. Cell. Biochem. 99: 978–985, 2006.

Key words: cdk10; splice variant; cell cycle; cdks

Cell division is regulated by a set of restriction points; the best characterized of these checkpoints are the G1/S and G2/M transitions. A family of serine/threonine protein kinases, referred to as cyclin-dependent kinases (cdks), currently consists of 12 members, cdks 1 through 12. They are termed cdks because they are inactive in their monomeric form and require association with a specific regulatory subunit, the cyclin partner, for activation. These kinases are responsible for the progression through the eukaryotic cell cycle and are also involved in transcriptional control, DNA repair, and post-mitotic events such as skeletal muscle and neuronal differentiation [MacLachlan et al., 1995; Morgan, 1995, 1997; Shuttleworth, 1995; Nasmyth, 1996; Sherr, 1996; Bagella et al., 1998; Price, 2000; Sano et al., 2002; Simone et al., 2002; Weishaupt et al., 2003; Chen et al., 2006].

Studies involving yeast represent the first model of the cell cycle. In *Saccharomyces cerevisiae*, cell division cycle 28 (CDC28) was found to be the only kinase involved in cell cycle progression [Nasmyth, 1993], while in *Schizosaccharomyces pombe*, the same function was observed to be carried out by cell division cycle 2 (cdc2) [Nurse and Bissett, 1981]. The vertebrate cell cycle proved to be a much more

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complex process. A human cdc2 homologue was identified by functional complementation of the *S. Pombe cdc2 mutation* [Lee and Nurse, 1987] and by antibody screening [Draetta et al., 1987], and was found to regulate, in association with cyclin B1, the onset of mitosis. Mammalian cdc2 (also called cdk1) did not control the G1/S transition; a new cdk was isolated (cdk2) and found to control the G1/S checkpoint [Elledge and Spottswood, 1991; Ninomiya-Tsuji et al., 1991; Tsai et al., 1991].

New members of the cdk family have been cloned based on homologies in sequences, and named according to their amino acid sequence in the conserved PSTAIRE domain. When they have a known cyclin partner, they should be renamed as cdks and be numbered in the order of their discovery.

PISSLRE, which has been renamed cdk10, although no associated cyclins have yet been found, was isolated based on two independent cloning strategies [Brambilla and Draetta, 1994; Grana et al., 1994]. Two different human cDNAs have been described to show a common region but to be divergent in their 5′- and 3′-ends, suggesting the possibility of differential expression of the human cdk10 gene (hcdk10). The two isoforms referred to by their GenBank entries as X78342 and L33264 are named hcdk10-1 and hcdk10-2, respectively, in this study, and they are derived from the same gene by alternative splicing [Crawford et al., 1999]. Although little is known about the proteins interacting with cdk10, hcdk10-2 has been proposed to be involved in the G2/M transition of the cell cycle [Li et al., 1995], while hcdk10-1 binds to Ets2 transcription factor, thereby modulating its transactivation activity [Kasten and Giordano, 2001]. A third human cdk10 isoform [GenBank AF153430] with few differences from hcdk10-1 has been isolated [Sergere et al., 2000] but no specific functions have yet been associated. These data suggest that the human cdk10 could be subjected to regulation by alternative splicing of its pre-mRNA. Alternative pre-mRNA splicing affects a multitude of human genes and plays important roles in development and disease. Variability in splicing patterns is a major source in expanding protein diversity and regulating gene expression in higher eukaryotes [Black, 2003; Lareau et al., 2004].

We cloned the mouse homologue of cdk10 (mcdk10). We identified a unique open reading frame coding 360 amino acids and sharing high homology with the human counterpart termed hcdk10-1. In an attempt to better understand cdk10's cellular functions we chose to test its role in cell cycle control and its association with Ets2 transcription factor in an analogous manner to its human counterparts. We found that murine cdk10 binds the Ets2 transcription factor and does not play a role in the G2/M phase of the cell cycle. In order to better characterize cdk10's function, future studies should focus on identifying the associated cyclins that interact with cdk10 and the putative substrates that could be phosphorylated by cdk10.

MATERIALS AND METHODS

cDNA Library Screening

To isolate the mouse homologue of the human cdk10 gene, a λ-zap mouse brain cDNA library (Stratagene, La Jolla, CA) was screened using a 1.1 kb human cdk10 cDNA fragment as a probe, as described [Sambrook et al., 1989]. Positive clones were plaque purified and the inserts were in vivo excised and circularized in XL1-blue *Escherichia coli* with an R408 helper phage (Stratagene). DNA sequencing was performed following the dideoxy-nucleotide chain termination [Sanger et al., 1977] using an Applied Biosystems Model 373 DNA sequencer. Both strands of mcdk10 cDNA were sequenced.

Northern Blot Analysis

Filter-immobilized polyadenylated RNA (2 μg/lane) from multiple adult murine tissues (Clontech, Palo Alto, CA) was hybridized to a ^{32}P-labeled mouse cdk10 cDNA probe. The membrane was pre-hybridized for 3 h at 42°C in a solution containing: 5× SSPE, 10× Denhardt's solution, 100 μg/ml of fresh denatured, sheared salmon sperm DNA, 50% formamide, 2% SDS and then hybridized for 12 h with a ^{32}P-dCTP random primer labeled cDNA probe using 1×10^6 cpm/ml. After overnight hybridization, blots were washed in 2× SSC, 0.2% SDS twice at room temperature for 10 min, and then in 0.1× SSC, 0.1% SDS three times at 42°C for 20 min and exposed to a Kodak X-ray film at −80°C with the aid of an intensifying screen.

Plasmids

Cdk10 mammalian expression construct (pcDNA3-HA-mcdk10wt) was created by fusing the hemaglutinin (HA) epitope to the carboxy-terminal of the full-length murine cdk10 in

pcDNA3 (Invitrogen). The dominant-negative counterpart (pcDNA3-HA-mcdk10dn) was generated by site-directed mutagenesis (QuickChange Site-directed Mutagenesis Kit, Stratagene), using pcDNA3-HA-mcdk10wt as a template, to replace aspartic acid 181 with an asparagine. The correct sequence of this construct was confirmed by sequencing using the Applied Biosystem model 373A DNA sequencer [Sanger et al., 1977]. The prokaryotic expression construct, pGEX 2T-mcdk10wt, was created by fusing the mcdk10wt segment to the glutathione-S-transferase of pGEX-2T (Pharmacia). The pcDNA3.1/HIS-Ets2 construct has been previously described [Kasten and Giordano, 2001]. The wild-type and dominant-negative cdc2 mammalian expression constructs were as previously described [van den Heuvel and Harlow, 1993].

In Vitro Binding Assay

One microgram of pcDNA3.1/HIS-Ets2 plasmid encoding wild-type Ets2 was in vitro translated according to the provided TNT rabbit reticulocyte lysate kit (Promega) with ^{35}S-methionine. Two microliters of the labeled sample was added to Glutathione/Sepharose beads coupled with 2 μg of GST proteins prepared as previously described [De Luca et al., 1997]. Incubation was carried out in NENT buffer (20 mM Tris-Cl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 % NP-40, 1 mM PMSF, and 10 μg/ml Leupeptin) for 2 h at 4°C with gentle rocking. Beads were washed three times in NENT buffer, and electrophoresis was performed on 10% acrylamide SDS–PAGE. Gels were dried and exposed at −70°C using Kodak Biomax MS films.

Cell Culture and Transient Transfections

The mouse embryo NIH/3T3 and the mouse L929 fibroblasts, as well as the human U-2 OS osteosarcoma cell line, were obtained from American Type Culture Collection (ATCC) (Rockville, MD) and cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 4 mM L-glutamine, 200 U/ml penicillin G sodium, and 200 mg/ml streptomycin sulfate in a 5% CO_2 atmosphere at 37°C. The cells were transfected in 100-mm culture dishes using a Polyfect/DNA mixture according to the manufacturer's instructions (Qiagen, Valencia, CA). Transfected cells were incubated at 37°C for 48 h after transfection and then collected for further analysis.

Colony Formation Assay

Exponentially growing cells (NIH/3T3, L929, U-2 OS) were transfected with 5 μg of pcDNA3-HA-mcdk10wt, pcDNA3-HA-mcdk10dn, CMV-cdc2wt, CMV-cdc2dn or the control vector. For selection, G418 (Gibco-BRL, Grand Island, NY) was added to the culture medium 48 h after transfection, at a final concentration of 800 μg/ml. After 6 days, 1×10^3 of G418-resistant cells in each well were plated in triplicate in 60-mm dishes, in order to evaluate their colony-forming ability. Cells were then incubated at 37°C for 8 days in a selecting medium containing G418 at a final concentration of 800 μg/ml. Colonies, defined as groups of a minimum of 50 cells, were counted after staining with 2% methylene blue in 95% ethanol.

Cell Cycle Analysis by Flow Cytometry

Cells (NIH/3T3, L929, U-2 OS) were transiently transfected with 5 μg of pcDNA3-HA-mcdk10wt, pcDNA3-HA-mcdk10dn, CMV-cdc2wt, CMV-cdc2dn or the control vector. A marker plasmid expressing enhanced green fluorescent protein (EGFP)-spectrin was included in the transfection mixture. Control cells, which were not transfected with EGFP-spectrin, were used as the EGFP-negative population. Cells were detached with trypsin and washed once with PBS. They were then fixed with ice-cold 70% ethanol at 4°C for 30 min and then washed once with PBS. Cells were then stained with 10 μg/ml Propidium Iodide (Roche Applied Science, Indianapolis, IN), 250 μg/ml RNase (Sigma, St. Louis, MO) in PBS and incubated at 37°C for 30 min in the dark. Transfected cells were gated according to their EGFP expression and the DNA content was determined by flow cytometry analysis. The percentage of cells in the different phases of the cell cycle was measured with a FACS calibur instrument (Becton-Dickinson, San Jose, CA) and the data obtained were analyzed by WinMDI 2.8 software.

RESULTS

Isolation and Characterization of the Mouse cdk10 cDNA

In order to isolate the mouse homologue of the human cdk10 gene, a mouse brain cDNA library

was screened at low stringency with a ~1 kb human cdk10 cDNA fragment. We chose a region spanning the identical sequence between the two human isoforms hcdk10-1 and hcdk10-2. Three positive clones were obtained and found to contain the same cDNA sequence, although the size of the inserts varied. When the sequence of the three different clones was compiled, it revealed a unique open reading frame through the entire length, from an ATG until a stop codon followed by 3′ untranslated sequences, including a poly-A tail. The complete sequence of mouse cdk10 cDNA is depicted in Figure 1. The length of the cDNA is 1,648 bp with a unique open reading frame from nucleotide 41 to a stop codon at nucleotide 1121. The murine cdk10 shows high similarity with the human counterpart hcdk10-1. They both show the translation initiation site (TIS) that begins with the codon ATG; hcdk10-2 begins with the codon GTG. They both encode 360 amino acids of the cdk10 protein with a predicted molecular weight of about 43 kDa. As depicted in Figure 2, the predicted amino acid sequence of the mouse cdk10 protein is highly homologous to the human protein cdk10-1, showing a high degree of conservation. Comparison of the amino acid sequences of the mouse and human cdk10 proteins shows that only 11 of 360 amino acids are different between the two counterparts, making the two proteins 96.9% identical.

Expression of cdk10 Gene in Mouse Tissues

A Northern blot analysis was performed on filter-immobilized polyadenylated RNA from multiple adult murine tissues (2 mg/lane) to analyze the expression pattern of the mouse cdk10 gene in adult mouse tissues. The mouse cdk10 gene was expressed as a 2.0 kb mRNA species in all tissues investigated (Fig. 3). Although cdk10 mRNA expression appeared to be ubiquitous, mRNA levels were higher in some tissues such as brain and liver. Interestingly, the level of mRNA transcript in the lung was very low.

Murine cdk10 and Ets2 Interact In Vitro

In order to investigate if mcdk10 was able to interact with Ets2, we performed coprecipitations of in vitro-translated ^{35}S-labeled Ets2 with GST-fusion proteins expressing murine cdk10, cdc2 or with GST alone. A strong interaction between Ets2 and cdk10 (Fig. 4, lane 4) was detected, while there was no interaction with the GST alone and GST-fusion protein expressing cdc2 (Fig. 4, lanes 2 and 3, respectively).

Our goal was also to determine whether cdk10 and Ets2 interact in mammalian cells. At present, however, we have not yet generated an antibody that recognizes mcdk10. We found that human cdk10 antibodies do not cross-react with the mouse counterpart by WB (data not shown). Nevertheless, we believe that mcdk10 interacts with Ets2 in vivo, analogously to human cdk10, which, moreover, inhibits Ets2 transactivation [Kasten and Giordano, 2001]. Whether or not the mcdk10 binds Ets2 in vivo, also modulating its transcriptional activity, needs to be clarified once the murine antibody has been generated.

Murine cdk10 Is Not Cell Cycle Related and Is Not Involved at the G2/M Checkpoint

The human counterpart hcdk10-2 is involved during the G2/M transition, analogously to cdc2. In order to analyze the role of mcdk10 during the cell cycle and during the G2/M checkpoint, a set of transfections was performed as described in the Materials and Methods and analyzed by colony formation assay and FACS analysis. Table I summarizes the results of the assays, indicating that mcdk10 does not perform any activity at the G2/M checkpoint and does not affect the proliferation rate of the cell lines analyzed, whereas cdc2dn, used as a positive control, shows a G2/M arrest. In addition, mcdk10 did not show any involvement in other cell cycle transitions that led us to conclude that mcdk10 is not cell cycle regulated. Moreover, this lack of activity could depend on the fact that the experiments were performed to express cdk10 alone, for which no regulatory subunits have yet been identified.

DISCUSSION

We isolated murine cdk10 (also referred to as PISSLRE) cDNA from a hybridization screening of a mouse brain library using a human cdk10 cDNA fragment as a probe. The sequence from each of the three independent clones obtained from the screening indicates that they all encode for the identical open reading frame. Although different isoforms of cdk10 have been identified in humans, we only isolated a single murine cdk10 cDNA. The mcdk10 putative ORF bears the strongest similarity to the hcdk10-1 isoform.

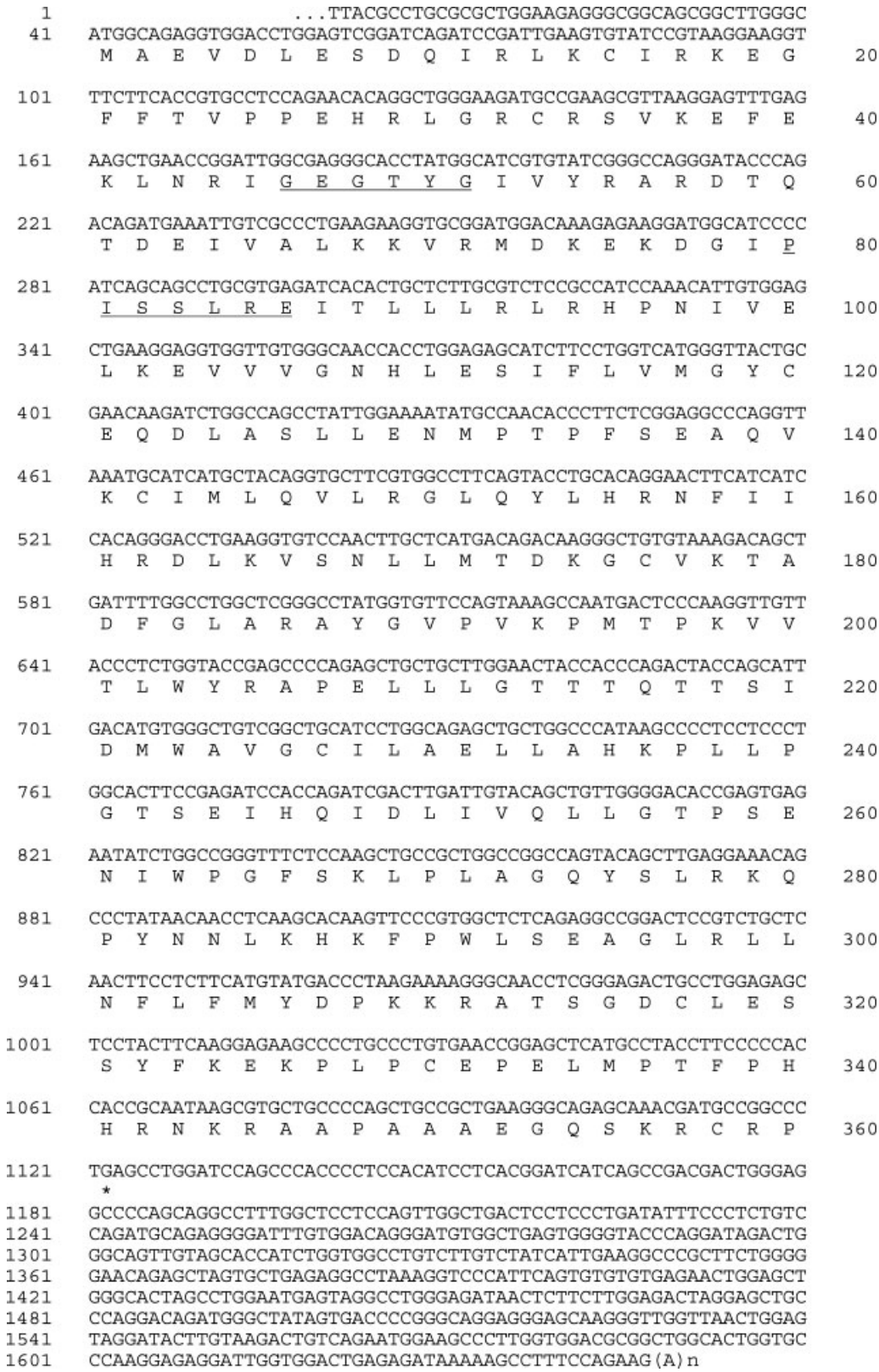

Fig. 1. Nucleotide sequence of mouse cdk10 cDNA and deduced amino acid sequence (in single-letter code). The putative ATP-binding site and the PSTAIRE-like motif are underlined. Nucleotides are numbered on the left, amino acids on the right.

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Mouse:   1 MAEVDLESDQIRLKCIRKEGFFTVPPEHRLGRCRSVKEFEKLNRIGEGTYGIVYRARDTQ  60
Human:   1 MAEPDLECEQIRLKCIRKEGFFTVPPEHRLGRCRSVKEFEKLNRIGEGTYGIVYRARDTQ  60

Mouse:  61 TDEIVALKKVRMDKEKDGIPISSLREITLLLRLRHPNIVELKEVVVGNHLESIFLVMGYC 120
Human:  61 TDEIVALKKVRMDKEKDGIPISSLREITLLLRLRHPNIVELKEVVVGNHLESIFLVMGYC 120

Mouse: 121 EQDLASLLENMPTPFSEAQVKCIMLQVLRGLQYLHRNFIIHRDLKVSNLLMTDKGCVKTA 180
Human: 121 EQDLASLLENMPTPFSEAQVKCIVLQVLRGLQYLHRNFIIHRDLKVSNLLMTDKGCVKTA 180

Mouse: 181 DFGLARAYGVPVKPMTPKVVTLWYRAPELLLGTTTQTTSIDMWAVGCILAELLAHKPLLP 240
Human: 181 DFGLARAYGVPVKPMTPKVVTLWYRAPELLLGTTTQTTSIDMWAVGCILAELLAHRPLLP 240

Mouse: 241 GTSEIHQIDLIVQLLGTPSENIWPGFSKLPLAGQYSLRKQPYNNLKHKFPWLSEAGLRLL 300
Human: 241 GTSEIHQIDLIVQLLGTPSENIWPGFSKLPLVGQYSLRKQPYNNLKHKFPWLSEAGLRLL 300

Mouse: 301 NFLFMYDPKKRATSGDCLESSYFKEKPLPCEPELMPTFPHHRNKRAAPAAAEGQSKRCRP 360
Human: 301 HFLFMYDPKKRATAGDCLESSYFKEKPLPCEPELMPTFPHHRNKRAAPATSEGQSKRCKP 360
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Fig. 2. Comparison of the predicted amino acid sequences of mouse cdk10 and the human homologue hcdk10-1 (see text). The difference between the mouse and human protein sequence is denoted by a bar between the two. The mouse sequence is on the top and the human sequence is on the bottom.

Alternative splicing is proposed to be responsible for the differences in the C-terminal translation products of the two human cdk10 transcripts, hcdk10-1 and hcdk10-2 [Crawford et al., 1999]. It is probable that the 134-bp sequence that is absent in hcdk10-2, but present in hcdk10-1, is the result of splicing at a non-consensus splice site. The absence of the 134-bp sequence leads to a shift in the reading frame and results in two alternative forms of human exon 14. As we state above, the mcdk10 cDNA is predicted to code for a protein with 96.9% identity to the human isoform hcdk10-1; however, the actual murine cDNA sequence excludes the possibility of differential splicing leading to an analogous hcdk10-2 isoform. In other words, none of the three possible reading frames derived from the mouse cDNA sequence is predicted to encode for a carboxy-terminal amino acid sequence similar to hcdk10-2. This observation may explain why we did not identify mouse cDNAs corresponding to an hcdk10-2 isoform even though we used a fragment spanning the common region of the two different isoforms in our library screening. It also suggests that mice may lack an orthologue of hcdk10-2 entirely.

Murine cdk10 is predicted to bear high identity to hcdk10-1 at the amino-terminal in addition to the carboxy-terminal. The genomic structure of the human cdk10 gene indicates that the hcdk10-1 transcript arises through exon scrambling [Crawford et al., 1999]. Exon scrambling has been previously described for mammalian genes where a pair of exons is joined at splice sites, but in an order different from the genomic DNA [Nigro et al., 1991;

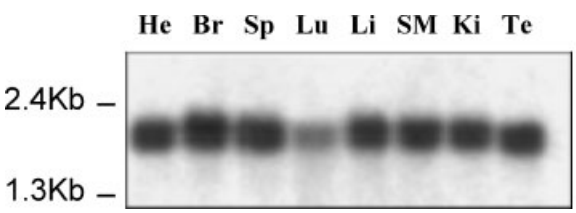

Fig. 3. Northern blot analysis of cdk10 expression in various mouse tissues. The size of molecular markers is indicated on the left. He, heart; Br, brain; Sp, spleen; Lu, lung; Li, liver; SM, skeletal muscle; Ki, kidney; Te, testis.

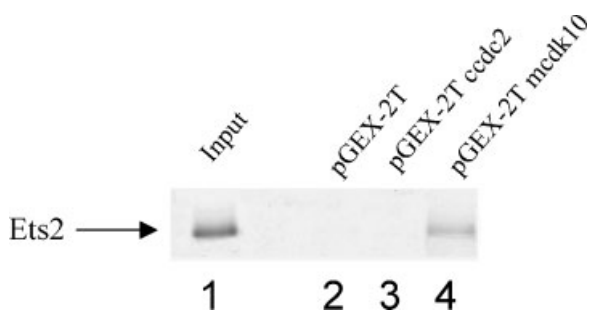

Fig. 4. Cdk10 interacts in vitro with Ets2. Equal amounts of GST and GST-fusion proteins of cdc2 and cdk10 were incubated with ^{35}S-labeled Ets2 in GST pull-down experiments. Recovered beads were resolved on 10% SDS polyacrylamide gel.

TABLE I. Cell Cycle and Colony Formation Analysis

Cell line	Phase	Vector	cdc2wt	cdc2dn	mcdk10wt	mcdk10dn
Cell cycle analysis						
	G0-G1	56.7	55.7	55.9	55.8	55.2
U2OS	S	37.7	39.4	31.4	38.8	39.1
	G2-M	5.6	4.9	12.7	5.8	5.7
	G0-G1	57	56.4	55.8	56.2	57.3
NIH3T3	S	34.8	36.3	30.2	35.4	35.2
	G2-M	8.2	7.3	14	8.4	7.5
	G0-G1	57.2	55.8	55.1	54.6	56.9
L929	S	37.4	38.5	31.1	38.7	37.8
	G2-M	5.4	5.7	13.8	6.7	5.3
Colony formation analysis						
U2OS		297	290	108	309	288
NIH3T3		289	301	126	292	281
L929		278	267	155	311	285

Percentages of cell in G0-G1, S, G2-M phases of the cell cycle and the values of the colony number are shown. Averages of duplicates from three independent experiments. SD <5%.

Cocquerelle et al., 1992; Zaphiropoulos, 1997; Caldas et al., 1998]. The scrambled hcdk10-1 transcript appears to constitute a small proportion of the human cdk10 mRNA, at least in the liver and the bladder carcinoma cell line examined [Crawford et al., 1999]. However, the fact that its murine counterpart was the only cdk10 transcript identified from a hybridization screening of a mouse brain cDNA library, and that it was obtained in three independent isolates suggests that this particular isoform does not make up just a minimal part of mouse cdk10. The genomic structure of mcdk10 is currently not known. The mouse transcript may undergo exon scrambling similar to its human orthologue; alternatively, the cdk10 gene may have undergone alterations in its genomic structure during the evolutionary split between humans and mice. Mouse and human cdk10 may differ in the order of their corresponding exons at the genomic level. If so, then the mcdk10 transcript may not be generated through exon scrambling at a non-consensus splice site and may explain the prevalence of the mouse transcript identified. Whether or not the mcdk10 transcript undergoes exon scrambling needs to be clarified once the mouse genome has been sequenced.

Human cdk10 isoforms show distinct physiological roles in cells. Hcdk10-1 is able to associate with the Ets2 transcription factor and inhibits its transactivation activity in mammalian cells [Kasten and Giordano, 2001]. Conversely, ectopic expression of an hcdk10-2 kinase inactive mutant arrests human cells in G2, suggesting that cdk10 may play a role in the cell cycle [Li et al., 1995]. In order to investigate the function of the mouse cdk10 transcript identified, we performed a set of experiments and found that mcdk10 binds Ets2 in vitro; however, we did not observe a G2 arrest when a mcdk10 kinase inactive mutant was overexpressed in three different mouse cell lines (NIH3T3, L929, and C2C12) analyzed. These observations suggest that different functional activities may be associated with alternative splicing in an analogous manner to the human counterparts. Indeed, mcdk10 may bind to Ets2 in vitro due to the high identity in sequence to the isoform hcdk10-1, while it lacks involvement at the G2/M checkpoint, since we did not isolate a mouse orthologue similar to hcdk10-2. Although it does not eliminate the possibility that mcdk10 has a role in the murine cell cycle, it is possible that mcdk10 lacks this cell cycle function because of the differences in the amino- and/or carboxy-terminals. Alternatively, human cells may have regulatory proteins that facilitate hcdk10-2′s G2 effect that are not present in the mouse, thereby leading to this gain of function by hcdk10-2. Further investigation of mouse cdk10 and its complexes will help to elucidate the various roles that this kinase plays in cells.

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REFERENCES

Bagella L, MacLachlan TK, Buono RJ, Pisano MM, Giordano A, De Luca A. 1998. Cloning of murine CDK9/PITALRE and its tissue-specific expression in development. J Cell Physiol 177:206–213.

Black DL. 2003. Mechanisms of alternative pre-messenger RNA splicing. Annu Rev Biochem 72:291–336.

Brambilla R, Draetta G. 1994. Molecular cloning of PISSLRE, a novel putative member of the cdk family of protein serine/threonine kinases. Oncogene 9:3037–3041.

Caldas C, So CW, MacGregor A, Ford AM, McDonald B, Chan LC, Wiedemann LM. 1998. Exon scrambling of MLL transcripts occur commonly and mimic partial genomic duplication of the gene. Gene 208:167–176.

Chen HH, Wang YC, Fann MJ. 2006. Identification and characterization of the CDK12/cyclin <1 complex involved in alternative splicing regulation. Mol Cell Biol 26(7):2736–2745.

Cocquerelle C, Daubersies P, Majerus MA, Kerckaert JP, Bailleul B. 1992. Splicing with inverted order of exons occurs proximal to large introns. EMBO J 11:1095–1098.

Crawford J, Ianzano L, Savino M, Whitmore S, Cleton-Jansen AM, Settasatian C, d'apolito M, Seshadri R, Pronk JC, Auerbach AD, Verlander PC, Mathew CG, Tipping AJ, Doggett NA, Zelante L, Callen DF, Savoia A. 1999. The PISSLRE gene: Structure, exon skipping, and exclusion as tumor suppressor in breast cancer. Genomics 56:90–97.

De Luca A, MacLachlan TK, Bagella L, Dean C, Howard CM, Claudio PP, Baldi A, Khalili K, Giordano A. 1997. A unique domain of pRb2/p130 acts as an inhibitor of Cdk2 kinase activity. J Biol Chem 272:20971–20974.

Draetta G, Brizuela L, Potashkin J, Beach D. 1987. Identification of p34 and p13, human homologs of the cell cycle regulators of fission yeast encoded by cdc2+ and suc1+. Cell 50:319–325.

Elledge SJ, Spottswood MR. 1991. A new human p34 protein kinase, CDK2, identified by complementation of a cdc28 mutation in Saccharomyces cerevisiae, is a homolog of Xenopus Eg1. EMBO J 10:2653–2659.

Grana X, Claudio PP, De Luca A, Sang N, Giordano A. 1994. PISSLRE, a human novel CDC2-related protein kinase. Oncogene 9:2097–2103.

Kasten M, Giordano A. 2001. Cdk10, a Cdc2-related kinase, associates with the Ets2 transcription factor and modulates its transactivation activity. Oncogene 20:1832–1838.

Lareau LF, Green RE, Bhatnagar RS, Brenner SE. 2004. The evolving roles of alternative splicing. Curr Opin Struct Biol 14:273–282.

Lee MG, Nurse P. 1987. Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2. Nature 327:31–35.

Li S, MacLachlan TK, De Luca A, Claudio PP, Condorelli G, Giordano A. 1995. The cdc-2-related kinase, PISSLRE, is essential for cell growth and acts in G2 phase of the cell cycle. Cancer Res 55:3992–3995.

MacLachlan TK, Sang N, Giordano A. 1995. Cyclins, cyclin-dependent kinases and cdk inhibitors: Implications in cell cycle control and cancer. Crit Rev Eukaryot Gene Expr 5:127–156.

Morgan DO. 1995. Principles of CDK regulation. Nature 374:131–134.

Morgan DO. 1997. Cyclin-dependent kinases: Engines, clocks, and microprocessors. Annu Rev Cell Dev Biol 13:261–291.

Nasmyth K. 1993. Control of the yeast cell cycle by the Cdc28 protein kinase. Curr Opin Cell Biol 5:166–179.

Nasmyth K. 1996. Viewpoint: Putting the cell cycle in order. Science 274:1643–1645.

Nigro JM, Cho KR, Fearon ER, Kern SE, Ruppert JM, Oliner JD, Kinzler KW, Vogelstein B. 1991. Scrambled exons. Cell 64:607–613.

Ninomiya-Tsuji J, Nomoto S, Yasuda H, Reed SI, Matsumoto K. 1991. Cloning of a human cDNA encoding a CDC2-related kinase by complementation of a budding yeast cdc28 mutation. Proc Natl Acad Sci USA 88:9006–9010.

Nurse P, Bissett Y. 1981. Gene required in G1 for commitment to cell cycle and in G2 for control of mitosis in fission yeast. Nature 292:558–560.

Price DH. 2000. P-TEFb, a cyclin-dependent kinase controlling elongation by RNA polymerase II. Mol Cell Biol 20:2629–2634.

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: A laboratory manual. New York: Cold Spring Harbor Laboratory Press.

Sanger F, Nicklen S, Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467.

Sano M, Abdellatif M, Oh H, Xie M, Bagella L, Giordano A, Michael LH, DeMayo FJ, Schneider MD. 2002. Activation and function of cyclin T-Cdk9 (positive transcription elongation factor-b) in cardiac muscle-cell hypertrophy. Nat Med 8:1310–1317.

Sergere JC, Thuret JY, Le Roux G, Carosella ED, Leteurtre F. 2000. Human CDK10 gene isoforms. Biochem Biophys Res Commun 276:271–277.

Sherr CJ. 1996. Cancer cell cycles. Science 274:1672–1677.

Shuttleworth J. 1995. The regulation and functions of cdk7. Prog Cell Cycle Res 1:229–240.

Simone C, Stiegler P, Bagella L, Pucci B, Bellan C, De Falco G, De Luca A, Guanti G, Puri PL, Giordano A. 2002. Activation of MyoD-dependent transcription by cdk9/cyclin T2. Oncogene 21:4137–4148.

Tsai LH, Harlow E, Meyerson M. 1991. Isolation of the human cdk2 gene that encodes the cyclin A- and adenovirus E1A-associated p33 kinase. Nature 353:174–177.

van den Heuvel S, Harlow E. 1993. Distinct roles for cyclin-dependent kinases in cell cycle control. Science 262:2050–2054.

Weishaupt JH, Neusch C, Bahr M. 2003. Cyclin-dependent kinase 5 (CDK5) and neuronal cell death. Cell Tissue Res 312:1–8.

Zaphiropoulos PG. 1997. Exon skipping and circular RNA formation in transcripts of the human cytochrome P-450 2C18 gene in epidermis and of the rat androgen binding protein gene in testis. Mol Cell Biol 17:2985–2993.