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Human and *Xenopus* MO15 mRNA are highly conserved but show different patterns of expression in adult tissues

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Abstract. Phosphorylation of human $p34^{cdc2}$ at Thr 161 seems to be necessary for its catalytic activity. CAK (cdk activating kinase) containing $p40^{MO15}$ from *Xenopus* egg extracts phosphorylates and activates $p34^{cdc2}$ in a cyclin dependent manner at Thr 161. We describe the cDNA sequence coding for human MO15, which predicts a serine/threonine kinase of 346 aa. Despite the high homology of 91% between the human and *Xenopus* proteins we observed a rather different mRNA distribution in adult tissues: In contrast to ubiquitously expressed human MO15-transcripts MO15-mRNA expression in *Xenopus* is restricted to oocytes indicating a different cellular role in these two phylogenetically distant species. By virtue of the homology to members of the family of cell cycle kinase genes we examined MO15 mRNA expression for its correlation to the proliferative activity of cells. Stimulation of lymphocytes showed MO15 mRNA expression to be independent of mitotic activity.

Introduction

Growth factors bind to specific cell surface molecules such as receptor-tyrosine kinases to initiate a signal transduction cascade that ultimately triggers the transcription of immediate and delayed early response genes. Late in G_1-phase growth factor induced signals activate the cell-cycle machinery, which contains cyclins and cyclin-dependend kinases (cdk) as key components (for a review see ref. 1). The protein cdk1 or $p34^{cdc2}$ was found to be the product of a cell division gene, cdc2 of *Schizosaccharomyces pombe* and CDC28 of *Saccharomyces cerevisiae* respectively. The kinase activity of these proteins is required for two key transitions in the cell cycle, namely the passage through Start and the G_2 to M transition.

The regulation of $p34^{cdc2}$ has been shown to be complex, involving subunit rearrangements as well as molecular modification. $p34^{cdc2}$ physically associates with cyclins, which are required for its activity, phosphorylation (2) and substrate specificity (3-6). The mitotic cyclins A and B were originally identified as proteins whose levels oscillate throughout the cell cycle (7). Connections between the cyclins as cell cycle regulators and cellular transformation provide evidence that disturbances in normal cell cycle control play a critical role in oncogenesis (for a review see ref. 8).

The reversible phosphorylation of proteins is recognized to be a major mechanism for the control of intracellular events in eukaryotic cells: Three major sites of phosphorylation (Thr 14, Tyr 15 and Thr 161) have been identified in the human $p34^{cdc2}$ protein. The dephosphorylation of Thr 14 and Tyr 15 as well as phosphorylation of Thr 161 are required for its activation. Negative regulation of mitosis occurs by phosphorylation of $p34^{cdc2}$ on Tyr 15 by the $wee1^+$ protein kinase with serine-, threonine- and tyrosine-phosphorylating activities (9,10). This leads to the inactivation of the $p34^{cdc2}$-cyclin B complex (10,11). Various observations suggest that the product of the cdc25 gene dephosphorylates Thr 14 and Tyr 15 thereby activating $p34^{cdc2}$ (for a review see ref. 12). The other phosphorylation of human $p34^{cdc2}$ which is necessary for its catalytic activity occurs at Thr 161 (corresponding to Thr 167 in *Schizosaccharomyces pombe*), a residue which is highly conserved in the cdk-family. Site-directed mutagenesis of this site provides a form of $p34^{cdc2}$ that lost its catalytic activity and can act in a dominant-negative fashion (13,14). In somatic cells of vertebrates and cell extracts from *Xenopus* eggs, formation of cyclin B-$p34^{cdc2}$ complexes requires phosphorylation of Thr 161 (15,16). Complexes of $p34^{cdc2}$ with cyclin B are inactivated at the transition from metaphase to anaphase. Subsequently, cyclins are degraded and Thr 161 is dephosphorylated (17). Thr 161 is located within the autophosphorylation domain of $p34^{cdc2}$ but neither $p34^{cdc2}$ alone nor complexes with cyclin B show autophosphorylation activity. In contrast, a kinase activity distinct from $p34^{cdc2}$ has been shown to phosphorylate Thr 161 in $p34^{cdc2}$ (16). CAK (cdk-activating kinase) from *Xenopus* egg extracts and starfish oocytes specifically phosphorylates and activates $p34^{cdc2}$ and $p33^{cdk2}$ in a cyclin-dependent manner at Thr 161 and Thr 160 respectively (18-21). In the purification procedure of CAK the starfish homologue of *Xenopus* $p40^{MO15}$ was found to remain associated to a second

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polypeptide. This peptide possibly is a regulatory subunit required to confer catalytic activity to $p40^{MO15}$ (19).

Protein-kinase genes are often expressed at low frequency and therefore difficult to analyze. This obstacle can be overcome by using degenerate oligonucleotide primers based on conserved motifs of the kinase domains for the PCR amplification of reverse-transcribed mRNA (22-26). In the present study we identified a gene coding for a protein kinase from embryonic tissues in a PCR-mediated approach. The cDNA representing the complete open reading frame, which is likely the human counterpart of MO15, was isolated from a cDNA library based on human RNA. In contrast to the *Xenopus* counterpart, the expression of human MO15 mRNA is ubiquitous in adult tissues suggesting a different role of MO15 in adult tissues of these phylogenetically distant species.

Materials and methods

DNA sequencing. 0.6 pmol of DNA, 4 pmol primer and a Taq DyeDeoxy Terminator Cycle Sequencing Kit from Applied Biosystems were used according to the manufacturer's protocol. The products of the sequencing reactions were purified by phenol/chloroform extraction and analyzed on an Applied Biosystem 373 A DNA sequencer. The primers used for amplification of the cDNA were ECO-VHRDL (5'-TTTGGAATTCGTNCAYMGNGAYYT-3') and P6[2DEA] (5'-TTGGAATTCATCCCNNNNNNCCACAC ATC-3') (23).

Amino acid sequences were aligned with the GAP program of HUSAR (Heidelberg UNIX Sequence Analysis Resource, Deutsches Krebsforschungszentrum, Heidelberg).

PCR. RT-PCR with RNA isolated from *Xenopus*-tissues was performed according to the following schedule: 95°C for 1 min, 60°C for 2 min and 72°C for 3 min and a final incubation for 10 min at 72°C.

RNA isolation and Northern blots. Xenopus-tissues were homogenized in a guanidinium isothiocyanate solution and RNA was isolated by centrifugation through a 5.7 M CsCl cushion. 20 µg total RNA was electrophoresed in a denaturing agarose/formaldehyde gel and transferred to a nitrocellulose membrane.

Purification of recombinant MO15-protein. GST-MO15 was constructed by cloning the PCR-amplified open reading frame of MO15 into the plasmid pGEX-2T (EcoRI/HindIII). Its orientation was checked by PCR and sequencing. Synthesis of recombinant proteins from transformed bacteria was induced with 100 µM isopropyl-ß-D-galactoside (IPTG) at 30°C for 16 h. After harvest and lysis of the cells the lysate was sonicated and centrifuged at 18.000 g for 30 min. Beads were collected by centrifugation and washed twice in lysis buffer. GST-MO15 was eluted with 5 mM reduced gluthathione (Sigma) in 50 mM Tris-HCl pH 8.0, 1 mM DTT. Samples were analyzed by 12% SDS-page. For thrombin digestion of GST-MO15, 1 µg of the fusion protein bound to glutathion-Sepharose was washed with buffer, digested with 10 µl of 10 U/ml thrombin at 28°C for 2 h and the supernatant was analyzed by SDS-page.

Screening of human cDNA libraries. An oligo(dT) primed cDNA library from a primary human squamous cell lung carcinoma in lambda gt10 was screened under high-stringency conditions (42°C, 50% formamide) with a probe corresponding to nt 514-666 of human MO15. A random primed cDNA library in lambda Max I from human thymus was screened under the same conditions with two probes corresponding to nt 514-666 and nt 4-262 of human MO15 respectively.

Results

Cloning and characterization of human MO15 cDNA. cDNA from human embryonic tissues was amplified using the primers (ECO-VHRDL and P6[2DEA]) corresponding to the highly conserved amino acid motifs from subdomains VI and IX (VHRDL and DVWXXG, respectively) of protein kinases (27). The PCR products were ligated into the Bluescript KSII(+) vector (Stratagene). Transformation of competent JM109 cells yielded 280 recombinant clones encompassing 7 unknown protein kinases. One of these clones was closely related to human cell cycle kinases such as cdk 1-3, PSSALRE and in particular to MO15 from *Xenopus*. This clone, designated K2, was used as a probe to screen 1.8×10^6 recombinant clones from an oligo(dT) primed cDNA library in lambda gt10 from a primary human squamous cell lung carcinoma and a random primed cDNA library in lambda Max I from human thymus. 5 clones ranging from 200 bp to 1040 bp were obtained. Determination of the nucleotide sequence of all overlapping clones and verification of the sequence by PCR and sequencing from normal tissue revealed a combined sequence of 1274 bp with a single open reading frame of 1038 nt ranging from a putative start codon at position 104 to an in-frame stop codon at position 1142 and predicting a protein of 39 kDa which contains 346 aa (Fig. 1). A stop codon in the same reading frame but upstream of the putative initiation codon was identified. We verified the predicted molecular weight of the K2-protein by using the *Escherichia coli* JM 109 strain and pGEX as vector to overexpress recombinant protein (Fig. 2).

A computer search indicated that the complete open reading frame of the putative K2-protein is homologous to a variety of protein kinases present in the Swissprot- and PIR-protein data base (EMBL) indicating that it belongs to this class of enzymes. Using the sequence of the complete open reading frame we determined the identity of the K2-protein to be 43% to the human $p34^{cdc2}$, 44.9% to cdk3, 46.4% to PSSALRE and 49.9% to $p33^{cdk2}$. The amino-terminal portion of the putative K2-polypeptide reveals conserved sequence motifs of serine/threonine kinases. The consensus sequence GXGXXG found in subdomain I (27) of nucleotide binding proteins and kinases is modified as GXGXXA-motif. In addition to the overall homology, domains to be highly conserved within the family of cdc-related kinases, such as the PSTAIRE motif or the residues whose phosphorylation appears to be significant for the activity of vertebrate cdc2-related gene products, are partially detectable in K2: 4 out of 7 aa from the PSTAIRE-motif are identical in K2. In contrast to Thr 161 of $p34^{cdc2}$, which has a corresponding residue in K2, the phosphorylatable residues Thr 14 and Tyr 15 were

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CCCGGTGGACGGAAGTGGGTGTTGGAGGCTTTAAGGTAGCTTTAAATTCGTGTTGTCCTG      60
GGAGCTCGCCCTTTTCGGCTGGAGTCGGGCTTTACGGCGCCGGATGGCTCTGGACGTGAA     120
                                                M  A  L  D  V  K     6

GTCTCGGGCAAAGCGTTATGAGAAGCTGGACTTCCTTGGGGAGGGACAGTTTGCCACCGT     180
  S  R  A  K  R  Y  E  K  L  D  F  L  G  E  G  Q  F  A  T  V      26

TTACAAGGCCAGAGATAAGAATACCAACCAAATTGTCGCCATTAAGAAAATCAAACTTGG     240
  Y  K  A  R  D  K  N  T  N  Q  I  V  A  I  K  K  I  K  L  G      46

ACATAGATCAGAAGCTAAAGATGGTATAAATAGAACCGCCTTAAGAGAGATAAAATTATT     300
  H  R  S  E  A  K  D  G  I  N  R  T  A  L  R  E  I  K  L  L      66

ACAGGAGCTAAGTCATCCAAATATAATTGGTCTCCTTGATGCTTTTGGACATAAATCTAA     360
  Q  E  L  S  H  P  N  I  I  G  L  L  D  A  F  G  H  K  S  N      86

TATTAGCCTTGTCTTTGATTTTATGGAAACTGATCTAGAGGTTATAATAAAGGATAATAG     420
  I  S  L  V  F  D  F  M  E  T  D  L  E  V  I  I  K  D  N  S     106

TCTTGTGCTGACACCATCACACATCAAAGCCTACATGTTGATGACTCTTCAAGGATTAGA     480
  L  V  L  T  P  S  H  I  K  A  Y  M  L  M  T  L  Q  G  L  E     126

ATATTTACATCAACATTGGATCCTACATAGGGATCTGAAACCAAACAACTTGTTGCTAGA     540
  Y  L  H  Q  H  W  I  L  H  R  D  L  K  P  N  N  L  L  L  D     146

TGAAAATGGAGTTCTAAAACTGGCAGATTTTGGCCTGGCCAAATCTTTTGGGAGCCCCAA     600
  E  N  G  V  L  K  L  A  D  F  G  L  A  K  S  F  G  S  P  N     166

TAGAGCTTATACACATCAGGTTGTAACCAGGTGGTATCGGGCCCCCGAGTTACTATTTGG     660
  R  A  Y  T  H  Q  V  V  T  R  W  Y  R  A  P  E  L  L  F  G     186

AGCTAGGATGTATGGTGTAGGTGTGGACATGTGGGCTGTTGGCTGTATATTAGCAGAGTT     720
  A  R  M  Y  G  V  G  V  D  M  W  A  V  G  C  I  L  A  E  L     206

ACTTCTAAGGGTTCCTTTTTTGCCAGGAGATTCAGACCTTGATCAGCTAACAAGAATATT     780
  L  L  R  V  P  F  L  P  G  D  S  D  L  D  Q  L  T  R  I  F     226

TGAAACTTTGGGCACACCAACTGAGGAACAGTGGCCGGACATGTGTAGTCTTCCAGATTA     840
  E  T  L  G  T  P  T  E  E  Q  W  P  D  M  C  S  L  P  D  Y     246

TGTGACATGTAAGAGTTTCCCTGGAATACCTTTGCATCACATCTTCAGTGCAGCAGGAGA     900
  V  T  C  K  S  F  P  G  I  P  L  H  H  I  F  S  A  A  G  D     266

CGACTTACTAGATCTCATACAAGGCTTATTCTTATTTAATCCATGTGCTCGAATTACGGC     960
  D  L  L  D  L  I  Q  G  L  F  L  F  N  P  C  A  R  I  T  A     286

CACACAGGCACTGAAAATGAAGTATTTCAGTAATCGGCCAGGGCCAACACCTGGATGTCA    1020
  T  Q  A  L  K  M  K  Y  F  S  N  R  P  G  P  T  P  G  C  Q     306

GCTGCCAAGACCAAACTGTCCAGTGGAAACCTTAAAGGAGCAAGCAAATCCAGCTTTGGC    1080
  L  P  R  P  N  C  P  V  E  T  L  K  E  Q  A  N  P  A  L  A     326

AATAAAAAGGAAAAGAACAGAGGCCTTAGAACAAGGAGGATTGCCCAAGAAACTAATTTT    1140
  I  K  R  K  R  T  E  A  L  E  Q  G  G  L  P  K  K  L  I  F     346

TTAAAGAGAACACTGGACAACATTTTACTACTGAGGGAAATAGCCAAAAAGGCAAATAAT    1200
GGAAAAATAGTAAACATTAAGTAAATGCTGTAGAAGTGAGTTTGTAAATATTCTACACAT    1260
GTAAAATATGTAAA                                                  1274
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Figure 1. Nucleotide and predicted amino acid sequences of human MO15 including partial untranslated sequences. The start codon is in bold type, stopcodons are underlined. The highly conserved motifs of the kinase domain (27) are shown in inverted letters: the invariant residues (DFG) implicated in ATP-binding and the consensus sequence GXGXXA of serine/threonine kinases related to cdc2 (33). The amino acids showing homology with the cdc2 PSTAIRE motif (34) are shaded.

not found in K2. This comparison demonstrates that K2 does not belong to the cdc2-family of polypeptides.

In contrast, homology of the K2-protein was found to be 86% identical and 91% similar (including conservative amino acid exchanges) to p40^{MO15} from *Xenopus* (18). Due to the high degree of homology and the same domain topology (Fig. 3), it seems very likely that K2 represents the human homologue of the *Xenopus* gene MO15. Therefore, we propose to call it MO15 as well. The gene was isolated in parallel by two groups (36,37) calling it (HS)CAK1 and STK1 respectively. Only very short stretches of starfish MO15 are known (19). These are highly conserved compared to the human counterpart. Most amino acid substitutions between human and *Xenopus* p40^{MO15} are located in the

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------MALDVKSRAKRYEKLDFLGEGQFATVYKARDKNT
MEGIAARGV..R....Q.....................

NQIVAIKKIKLGHRSEAKDGINRTALREIKLLQELSHPNI
DR...........A..N.....................

IGLLDAFGHKSNISLVFDFMETDLEVIIKDNSLVLTPSHI
.............................T......A..

KAYMLMTLQGLEYLHQHWILHRDLKPNNLLLDENGVLKLA
.S............HL.....................

DFGLAKSFGSPNRAYTHQVVTRWYRAPELLFGARMYGVGV
............I..........S.............

DMWAVGCILAELLLRVPFLPGDSDLDQLTRIFETLGTPTE
....................................

EQWPDMCSLPDYVTCKSFPGIPLHHIFSAAGDDLLDLIQG
....G.S.....AF.....T...L..I.......E.L..

LFLFNPCARITATQALKMKYFSNRPGPTPGCQLPRPNCPV
..T......C..S...RKR......A....NL......SI

ETLKEQANPALAIKRKRTEALEQGGLPKKLIF
.A....Q.LN.G.......GMD.KDIA...S.
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Figure 2. Deduced amino acid sequence of human MO15 and its comparison with the *Xenopus* homologue. Only the residues differing between human and *Xenopus* sequences are shown for the latter. Gaps are indicated by hyphens.

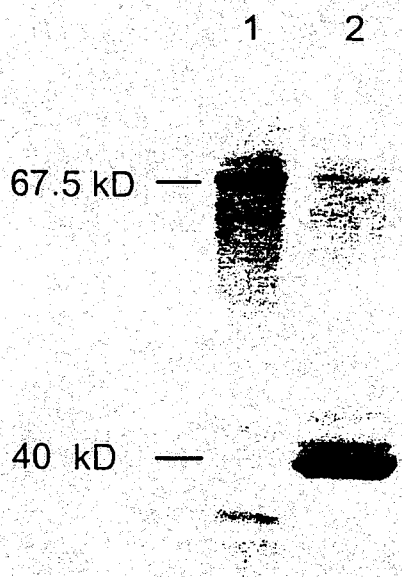

Figure 3. Expression and purification of recombinant MO15-protein from bacteria. SDS-page of GST-MO15 (lane 1) and GST-MO15 after thrombin digestion (lane 2).

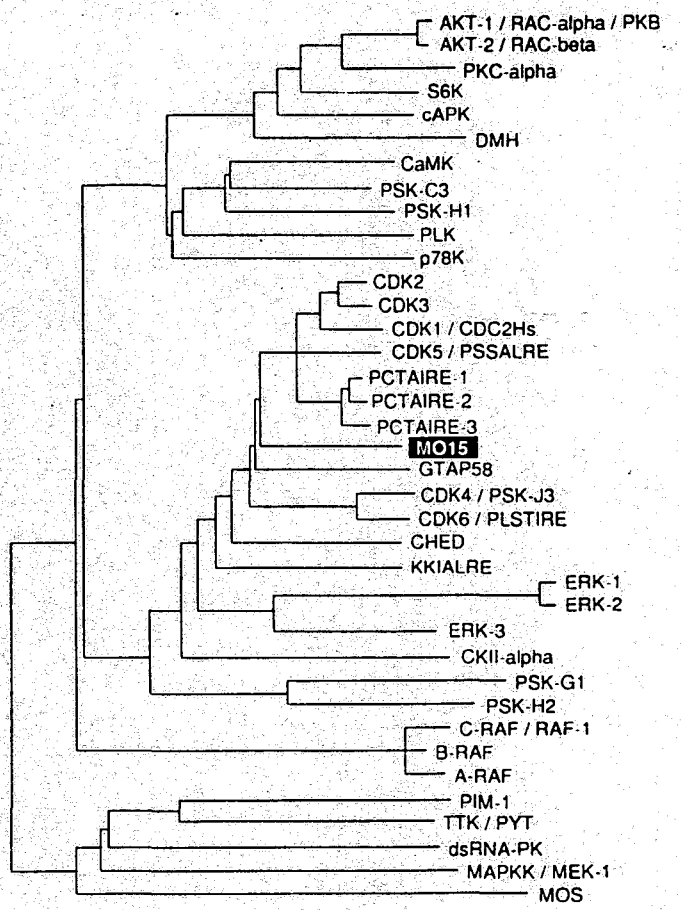

Figure 4. Phylogenetic relationship of 38 human serine/threonine kinase sequences. The sequences were obtained from the Protein Kinase Data Bank of S. Hanks *et al* (The Salk Institute) and the EMBL, Genbank, Swissprot and PIR databases. 38 human serine/threonine kinase sequences were aligned over the entire catalytic domains (27). Phylogenetic relationships were determined by using the TREE program of HUSAR (Heidelberg UNIX Sequence Analysis Resource, Deutsches Krebsforschungszentrum, Heidelberg), based on the progressive alignment method of Feng and Doolittle (35) in a multiple sequence alignment.

carboxyterminal portion of the protein. Moreover, the aminoterminus of $p40^{MO15}$ from *Xenopus* is by 6 aa longer than the human polypeptide. The ATP-binding domain of the MO15 protein is identical compared to its starfish and *Xenopus* counterparts (18,19). The following motifs in subdomains VI (HRDLKPN, aa 135-141) and VIII (GSPNRAYTH, aa 163-171), which are characteristic for certain serine/threonine kinases, are almost identical between the counterparts of MO15.

These data indicate that MO15 is a gene highly conserved through evolution from echinoderms to mammalia. The sequences of the catalytic domains of 38 human serine/threonine kinases were used to determine their phylogenetic relationships. As shown in Fig. 4 $p34^{cdc2}$-related molecules fall into a number of sub-families. For example, the MO15-protein is most closely related to the PCTAIRE-subgroup and to CDK1-3, which share the highly conserved PSTAIRE-motif. Comparing MO15- with the CDK-family and PCTAIRE-subgroup-proteins we find a homology between 39 and 47%.

Human and Xenopus MO15 mRNA show diverging patterns of expression in adult tissues. Using a DNA fragment of MO15 which overlaps with its kinase domain as a probe we performed a Northern blot analysis of RNAs from a variety of human tissues. Transcripts of 1.8 kb were detected in all tissues tested (Fig. 5a,b). Very high levels of transcripts were observed in placenta, skeletal muscle, kidney, testis, ovary, and colon. Regarding the fact that the catalytic domains of protein-kinases are highly conserved (27) we could not rule

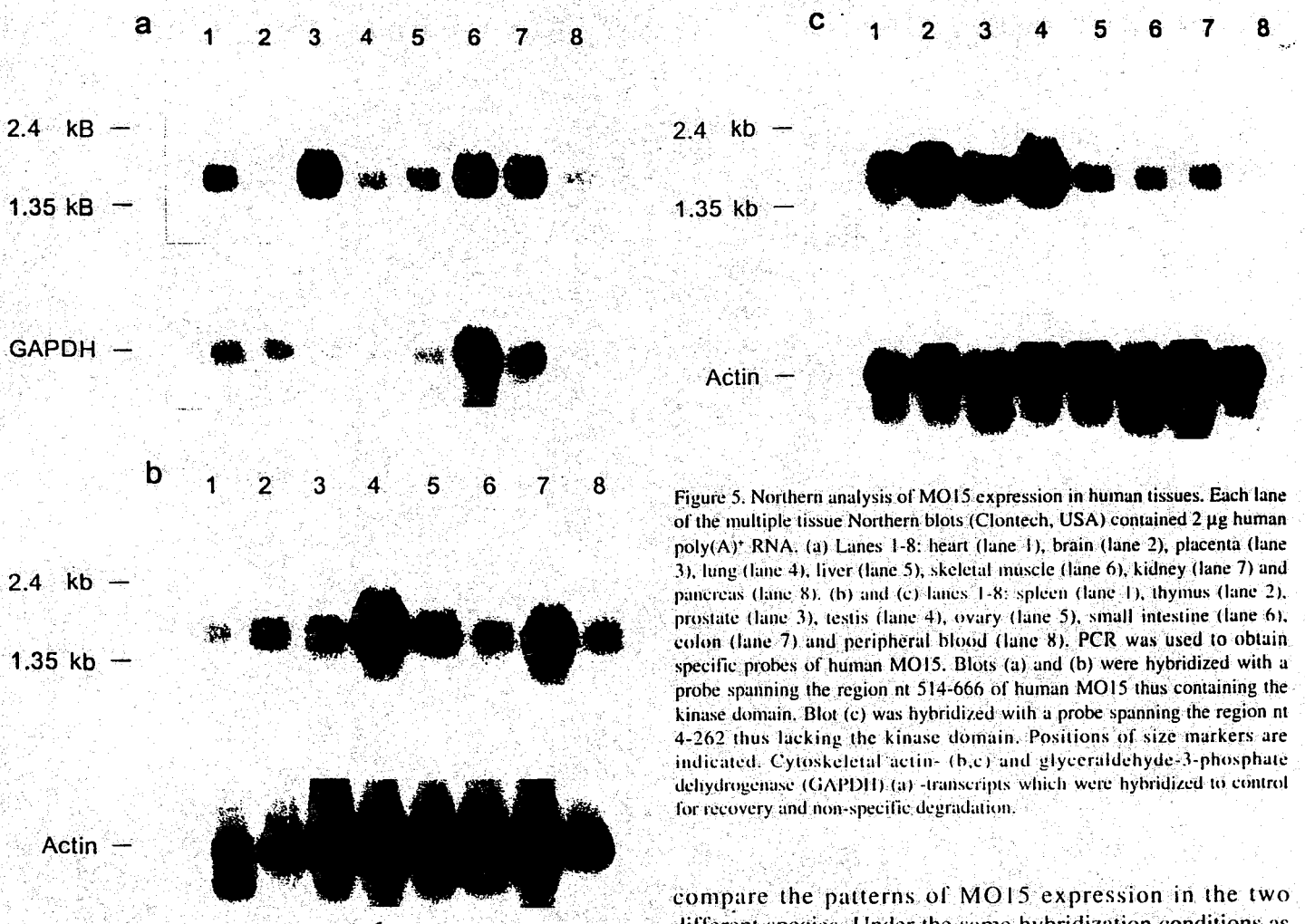

Figure 5. Northern analysis of MO15 expression in human tissues. Each lane of the multiple tissue Northern blots (Clontech, USA) contained 2 μg human poly(A)$^+$ RNA. (a) Lanes 1-8: heart (lane 1), brain (lane 2), placenta (lane 3), lung (lane 4), liver (lane 5), skeletal muscle (lane 6), kidney (lane 7) and pancreas (lane 8). (b) and (c) lanes 1-8: spleen (lane 1), thymus (lane 2), prostate (lane 3), testis (lane 4), ovary (lane 5), small intestine (lane 6), colon (lane 7) and peripheral blood (lane 8). PCR was used to obtain specific probes of human MO15. Blots (a) and (b) were hybridized with a probe spanning the region nt 514-666 of human MO15 thus containing the kinase domain. Blot (c) was hybridized with a probe spanning the region nt 4-262 thus lacking the kinase domain. Positions of size markers are indicated. Cytoskeletal actin- (b,c) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (a) -transcripts which were hybridized to control for recovery and non-specific degradation.

out that the data presented in Figs. 5a and b are at least partially due to cross-hybridization. Thus, a region 5' from the kinase domain which is known to be more divergent within the family of protein kinases was chosen for the generation of a second probe. Still, MO15 transcripts were found in all human tissues analyzed (Fig. 5c). Comparing Figs. 5b and c which contain the same RNA but differ in the probe used we observe diverging patterns of hybridization under high stringency conditions. This suggests that in human tissues MO15-related transcripts are present. Thus, an influence by different thermodynamic stabilities of the two probes used or by alternative splicing of the MO15-message, as shown by Levedakou *et al* (36), can not be ruled out.

In contrast to our data on human adult tissues *Xenopus* MO15 transcripts were not detected in adult tissues and were found to accumulate only during oogenesis (18). We therefore analyzed RNA from various *Xenopus* tissues to compare the patterns of MO15 expression in the two different species. Under the same hybridization conditions as applied for the Northern blots shown in Fig. 5 we detected MO15 transcripts only in *Xenopus* oocytes (Fig. 6). An identical hybridization pattern was obtained with probes from the aminoterminal or the kinase domain (Fig. 6a and b). Only in oocytes we found two MO15-related transcripts under conditions of high resolution (Fig. 6a), which differ by approximately 100 bp. As shown in Fig. 7a, RT-PCR with RNA from *Xenopus* confirmed the high expression in oocytes. However, with increased sensitivity due to additional PCR cycles (Fig. 7b), weak signals also appeared in lung, ovary, and colon.

In view of the fact that for a gene from two very distant phylogenetic species the homology of MO15 is very high, conservation of the structure and function of $p40^{MO15}$ seems to be crucial for its role in cellular physiology. Therefore, the fundamental difference in the pattern of expression of this gene in adult tissues is unexpected and quite remarkable.

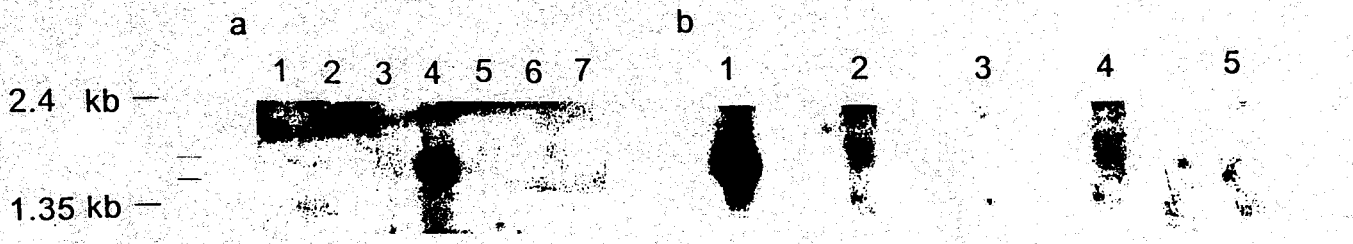

Figure 6. Northern analysis of MO15 expression in *Xenopus* laevis tissues. (a) RNA from heart (lane 1), liver (lane 2), colon (lane 3), oocytes (lane 4), ovary (lane 5), lung (lane 6) and muscle (lane 7) was hybridized with a ^{32}P-labelled probe starting at codon 76 and ending at codon 196 of the *Xenopus* MO15 sequence (18). (b) RNA from oocytes (lane 1), ovary (lane 2), colon (lane 3), liver (lane 4) and heart (lane 5) was hybridized with a ^{32}P-labelled probe starting at codon 515 and ending at codon 685, thus containing the kinase domain of the *Xenopus* MO15 sequence (18).

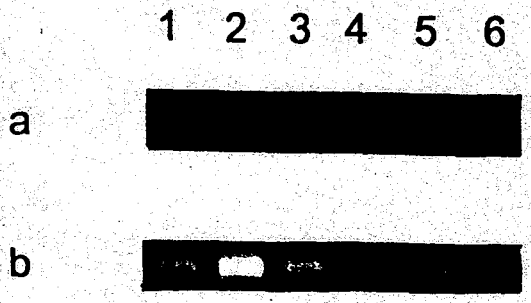

Figure 7. Expression of *Xenopus* laevis MO15 in different adult tissues and oocytes. RT-PCR was used to analyze the expression of MO15 mRNA. The source of the RNAs for the RT reactions is indicated above each lane. 1=lung, 2=oocytes, 3=ovary, 4=muscle, 5=colon, 6=liver. (a) 30 cycles, (b) 34 cycles.

Expression of MO15 mRNA in resting and mitogen-induced cells. Since MO15 clearly belongs to the family of cell cycle-kinases we wondered if the expression of MO15 correlates with the proliferative activity of cells. Lymphocytes from human peripheral blood (PBL) were grown in presence of phytohemagglutinine (PHA) and interleukin 2 (IL-2) to induce proliferation. PHA activates the expression of IL-2 and its high-affinity receptor, which are components of an autocrine loop leading to lymphocyte proliferation.

PHA was added to lymphocytes in culture at day 0 and removed at day 2. RNA from these lymphocytes collected at days 0, 1 and 2 was analyzed for MO15 expression in Northern blot experiments (Fig. 8a, lanes 1-3). After removal of PHA lymphocytes were cultivated for three additional days (Fig. 8a, lane 7-9). Furthermore, cycloheximide, an inhibitor of protein synthesis, was used to study the effect of *de novo* protein synthesis on the level of MO15 transcripts. The results show that resting cells express MO15 mRNA (Fig. 8b, lane 1) and that the PHA-induced autocrine IL-2 stimulation does not change the level of MO15 expression (Fig. 8a, lanes 7-9). Subsequent activation of lymphocytes with IL-2 does not alter the level of MO15 expression either (Fig. 8a, lanes 4-6). Thus, the mRNA expression of human MO15 does not seem to be associated with the mitotic activity of cells. Furthermore cycloheximide does not influence MO15 mRNA expression in lymphocytes (Fig. 8b, lanes 1-4).

Discussion

The pattern of expression of MO15 in human tissues is totally different from the expression of the homologous gene in *Xenopus*: No MO15 transcripts could be detected in adult tissues from *Xenopus* such as adult liver, spleen, testis or skeletal muscle. *Xenopus* MO15 mRNA is accumulated during oogenesis, becomes deadenylated during meiotic maturation and is degraded after the mid-blastula-transition state of embryogenesis. Shuttleworth *et al* (18) draw the conclusion that the function of *Xenopus* $p40^{MO15}$ correlates with processes before or during maturation of oocytes. Thus, $p40^{MO15}$ would either control arrest of meiosis or negatively trigger the events leading to the activation of MPF. In contrast, human MO15 appears to be expressed ubiquitously in adult tissues. Due to this diverging pattern of expression of human and *Xenopus* MO15 we would like to suggest, that MO15 has functions in the mammalian cell which either are not needed in amphibia or taken over by other genes in these species.

Interestingly, such differences in expression also seem to apply to other genes: c-*myb* was found in a broad spectrum of tissues from *Xenopus* (intestine, liver, heart, lung, ovary, kidney, spleen, oviduct, testis and blood) (28), while human c-*myb*-mRNA seems to be restricted to cells of hematopoietic origin (29). Also, mouse c-*myb* mRNA was detected by Northern blot hybridization only in lymphopoietic tissues (spleen and thymus) (30). c-*mos* transcripts are detectable by Northern analysis in testis, ovary and brain of *Xenopus* (31). Using Northern blots *mos* mRNA was found in testis and ovary of mouse and furthermore in brain, kidney, placenta, mammary gland, heart and lung by applying the S1 nuclease protection assay (32). Various groups applied techniques of different sensitivity to evaluate the mRNA expression of c-*mos* and c-*myb*, therefore, it is hard to estimate if the patterns of expression diverge in a similar fashion as shown for MO15.

Many groups have shown that *Xenopus* and starfish MO15 is the catalytic subunit of a protein kinase that activates $p34^{cdc2}$ (18,19,21). Meyerson *et al* (33) compared the distribution of cdk-related protein kinase-mRNAs in human tissues. In this study, cdc2-transcripts were only detected in human lung and placenta, but none in heart, brain, liver, skeletal muscle, kidney and pancreas. It is surprising

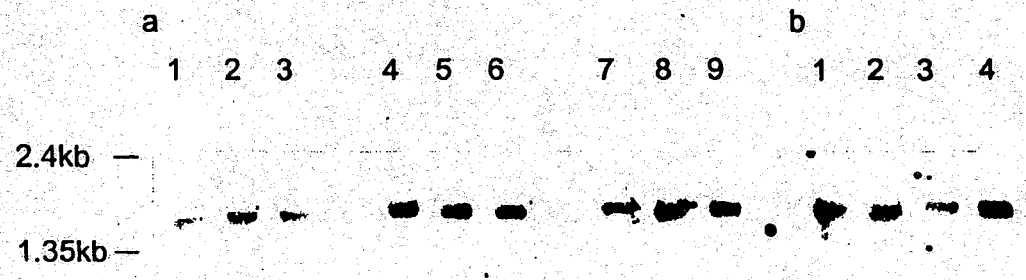

Figure 8. Influence of PHA, IL-2 and cycloheximide on MO15 mRNA expression in human peripheral blood lymphocytes. Northern blot analysis of RNA from human lymphocytes with a MO15-specific probe. Each lane contained 7 µg of total RNA. The blot was hybridized with a probe spanning the region nt 514-666 of human MO15 thus containing the kinase domain. Size markers are indicated. (a) MO15 mRNA expression in: resting lymphocytes (lane 1), lymphocytes stimulated with PHA for 1 day (lane 2) and 2 days (lane 3), in lymphocytes stimulated with PHA for 3 days and then cultured without PHA for 1 (lane 7), 2 (lane 8) and 3 (lane 9) additional days. MO15 mRNA expression in: lymphocytes stimulated with PHA for 3 days and after removal of PHA cultured with IL-2 for 1 (lane 4), 2 (lane 5) or 3 days (lane 6). (b) MO15 mRNA expression in: resting lymphocytes (lane 1), lymphocytes stimulated with PHA for 1 day (lane 2), stimulated with PHA for 2 days (lane 3) and stimulated for 1 day with PHA and cultured 1 additional day with cycloheximide and PHA (lane 4).

that mRNA coding for $p40^{MO15}$ the putative regulator of $p34^{cdk2}$-activity is present in tissues which are missing its target $p34^{cdc2}$. Meyerson *et al* (33) present a broad spectrum of expression data of various cdc-related genes. Like MO15 members of this family, such as cdk2, PSSALRE, PCTAIRE-1 and PCTAIRE-3 show a broad range of expression in human tissues. Possibly, therefore $p40^{MO15}$ activates targets other than $p34^{cdc2}$. This has been shown already for $p33^{cdk2}$ (21), whose transcripts show a pattern of expression which is very similar to MO15.

Our data indicate that the expression of human MO15 does not correlate with the meiotic or mitotic activity of cells. Despite a very high identity of human and *Xenopus* MO15-proteins their function in those phylogenetically distant species seems to be of fundamental difference.

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References

1. Norbury C and Nurse P: Animal cell cycles and their control. Annu Rev Biochem 61: 441-470, 1992.
2. Draetta G, Luca F, Westendorf J, Brizuela L, Ruderman J and Beach D: cdc2 protein kinase is complexed with both cyclin A and B: evidence for proteolytic inactivation of MPF. Cell 56: 829-838, 1989.
3. Murray AW and Kirschner MW: Cyclin synthesis drives the early embryonic cell cycle. Nature 339: 275-280, 1989.
4. Peter M, Nakagawa J, Doree M, Labbe JC and Nigg EA: *In vitro* disassembly of the nuclear lamina and M phase-specific phosphorylation of lamins by cdc2 kinase. Cell 61: 591-602, 1990.
5. Solomon MJ, Glotzer M, Lee TH, Philippe M and Kirschner MW: Cyclin activation of p34 *cdc*2. Cell 63: 1013-1024, 1990.
6. Sherr CJ: Mammalian G_1 cyclins. Cell 73: 1059-1065, 1993.
7. Evans T, Rosenthal ET, Youngblom J, Distel D and Hunt T: Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. Cell 33: 389-396, 1983.
8. Hunter T and Pines J: Cyclins and cancer. Cell 66: 1071-1074, 1991.
9. Featherstone C and Russel P: Fission yeast $p107^{wee1}$ mitotic inhibitor is a tyrosine/serine kinase. Nature 349: 808-811, 1991.
10. Parker LL, Atherton-Fessler S and Piwnica-Worms H: $p107^{wee1}$ is a dual-specificity kinase that phosphorylates $p34^{cdc2}$ on tyrosine 15. Proc Natl Acad Sci USA 89: 2917-2921, 1992.
11. McGowan CH and Russell P: Human Wee1 kinase inhibits cell division by phosphorylating $p34^{cdc2}$ exclusively on Tyr15. EMBO J 12: 75-85, 1993.
12. Millar JBA and Russell P: The cdc25 M-phase inducer: An unconventional protein phosphatase. Cell 68: 407-410, 1992.
13. Gould KL, Moreno S, Owen DJ, Sazer S and Nurse P: Phosphorylation at Thr 167 is required for *Schizosaccharomyces pombe* $p34^{cdc2}$ function. EMBO J 10: 3297-3309, 1991.
14. Fleig UN, Gould KL and Nurse P: A dominant negative allele of $p34^{cdc2}$ shows altered phosphoamino acid content and sequesters $p56^{cdc13}$ cyclin. Mol Cell Biol 12: 2295-2301, 1992.
15. Krek W and Nigg EA: Differential phosphorylation of vertebrate $p34^{cdc2}$ kinase at the G_1/S and G_2/M transitions of the cell cycle: identification of major phosphorylation sites. EMBO J 10: 305-316, 1991.
16. Solomon MJ, Lee T and Kirschner MW: Role of phosphorylation in $p34^{cdc2}$ activation: Identification of an activating kinase. Mol Cell Biol 3: 13-27, 1992.
17. Lorca T, Labbe JC, Devault A, Fesquet D, Capony JP, Cavadore JC, Le Bouffant F and Doree M: Dephosphorylation of cdc2 on Threonine 161 is required for kinase inactivation and normal anaphase. EMBO J 11: 2381-2390, 1992.
18. Shuttleworth J, Godfrey R and Colman A: A cdc2-related protein kinase involved in negative regulation of meiotic maturation of *Xenopus* oocytes. EMBO J 9: 3233-3240, 1990.
19. Fesquet D, Labbe JC, Derancourt J, Capony JP, Galas S, Girard F, Lorca T, Shuttleworth J, Doree M and Cavadore JC: The MO15 gene encodes the catalytic subunit of a protein kinase that activates cdc2 and other cyclin-dependent kinases (CDKs) through phosphorylation of Thr161 and its homologues. EMBO J 12: 3111-3121, 1993.
20. Solomon MJ, Harper JW and Shuttleworth J: CAK, the $p34^{cdc2}$ activating kinase, contains a protein identical or closely related to $p40^{MO15}$. EMBO J 12: 3133-3142, 1993.
21. Poon RYC, Yamashita K, Adamczewski JP, Hunt T and Shuttleworth J: The cdc2-related protein $p40^{MO15}$ is the catalytic subunit of a protein kinase that can activate $p33^{cdk2}$ and $p34^{cdc2}$. EMBO J 12: 3123-3132, 1993.
22. Holtrich U, Bräuninger A, Strebhardt K and Rübsamen-Waigmann H: Two additional protein-tyrosine kinases expressed in human lung: Fourth member of the fibroblast growth factor receptor family and an intracellular protein-tyrosine kinase. Proc Natl Acad Sci USA 88: 10411-10415, 1991.
23. Holtrich U, Wolf G, Bräuninger A, Karn T, Böhme B, Rübsamen-Waigmann H and Strebhardt K: Induction and down-regulation of PLK, a human serine/threonine kinase expressed in proliferating cells and tumors. Proc Natl Acad Sci USA 91: 1736-1740, 1994.
24. Bräuninger A, Holtrich U, Strebhardt K and Rübsamen-Waigmann H: Isolation and characterization of a human gene that encodes a new subclass of protein tyrosine kinases. Gene 110: 205-211, 1992.
25. Böhme B, Holtrich U, Wolf G, Luzius H, Grzeschik HK, Strebhardt K and Rübsamen-Waigmann H: PCR mediated detection of a new human receptor-tyrosine-kinase, HEK2. Oncogene 8: 2857-2862, 1993.
26. Karn T, Holtrich U, Bräuninger A, Böhme B, Wolf G, Rübsamen-Waigmann H and Strebhardt K: Structure, expression and chromosomal mapping of TKT from man and mouse: a new subclass of receptor tyrosine kinases with a factor VIII-like domain. Oncogene 8: 3433-3440, 1993.
27. Hanks SK, Quinn AM and Hunter T: The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science 241: 42-52, 1988.
28. Amaravadi L and King MW: Characterization and expression of the *Xenopus* c-Myb homolog. Oncogene 9: 971-974, 1994.
29. Westin EH, Gallo RC, Arya SK, Eva A, Souza LM, Baluda MA, Aaronson SA and Wong-Staal F: Differential expression of the *amv* gene in human hematopoietic cells. Proc Natl Acad Sci USA 79: 2194-2198, 1982.
30. Mushinski JF, Potter M, Bauer SR and Premkumar Reddy E: DNA rearrangement and altered RNA expression of the c-*myb* oncogene in mouse plasmacytoid lymphosarcomas. Science 220: 795-798, 1983.
31. Sagata N, Oskarsson M, Copeland T, Brumbaugh J and Vande Woude GF: Function of c-*mos* proto-oncogene product in meiotic maturation in *Xenopus* oocytes. Nature 335: 519-525, 1988.
32. Propst F, Rosenberg MP, Iyer A, Kaul K and Vande Woude GF: c-*mos* proto-oncogene RNA transcripts in mouse tissues: Structural features, developmental regulation and localization in specific cell types. Mol Cell Biol 7: 1629-1637, 1987.
33. Meyerson M, Enders GH, Wu CL, Su LK, Gorka C, Nelson C, Harlow E and Tsai LH: A family of human cdc2-related protein kinases. EMBO J 11: 2909-2917, 1992.
34. Lee MG and Nurse P: Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2. Nature 327: 31-35, 1987.
35. Feng D-F and Doolittle RF: Progressive sequence alignment as a prerequisite to correct phylogenetic trees. J Mol Evol 25: 351-360, 1987.
36. Levedakou EN, He M, Baptist EW, Craven RJ, Cance WG, Welcsh PL, Simmons A, Naylor SL, Leach RJ, Lewis TB, Bowcock A and Liu ET: Two novel human serine/threonine kinases with homologies to the cell cycle regulating *Xenopus* MO15 and NIMA kinases: cloning and characterization of their expression pattern. Oncogene 9: 1977-1988, 1994.
37. Wu L, Yee A, Liu L, Carbonaro-Hall D, Venkatesan N, Tolo VT and Hall FL: Molecular cloning of the human CAK1 gene encoding a cyclin-dependent kinase-activating kinase. Oncogene 9: 2089-2096, 1994.