# Human SAK related to the PLK/polo family of cell cycle kinases shows high mRNA expression in testis

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Abstract. We identified the nucleotide sequence of a cDNA encoding a polypeptide with a kinase domain that is related to the catalytic region of Drosophila melanogaster polo, Saccharomyces cerevisiae CDC5 as well as human FNK and PLK. The novel gene seems to represent the human counterpart of the mouse gene sak. The sequence of SAK predicts a serine/threonine kinase of 970 aa. The distribution of SAK mRNA in adult organs is restricted to certain tissues such as testis and thymus. Northern analyses of tumor tissues (lung, breast, brain) and corresponding normal tissues from the same patient did not reveal SAK expression. Comparing the mRNA distribution of the proliferation-associated pololike kinase (PLK) with the expression of SAK we observed distinct differences. Thus, we suggest that these kinases have unique physiological roles in different cells or in response to different signals.

## Introduction

Phosphorylation plays a pivotal role in controlling the progression through the eukaryotic cell cycle, cellular differentiation and changes of cellular structures. The *Drosophila melanogaster polo* gene (1) and the *Saccharomyces cerevisiae* cell cycle gene CDC5 (2) are two conserved protein kinases which are required for progression through mitosis: Mutations in polo result in abnormal chromosome segregation in larval neuroblasts of *Drosophila* due to defective spindel formation in mitotic and meiotic cells (1,3). Deletion of CDC5 was lethal in *Saccharomyces cerevisiae* displaying an abnormal morphology of dividing cells with their nuclei almost divided but still connected (2). Recently, two human serinc/threonine kinases have been identified

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which are homologous to polo: PLK (4-7) and FNK/PRK (8,9, Holtrich *et al*, unpublished). It could be demonstrated that PLK mRNA expression is regulated during terminal erythrodifferentiation and during the cell cycle (4). In our own studies several lines of evidence indicate that the levels of PLK-mRNA and -protein correlated with cellular proliferation (7,10). In addition we found that the prognosis of patients suffering from non-small cell lung cancer (NSCLC) correlates with PLK mRNA expression in lung tumor tissues. Thus, the determination of PLK mRNA expression helps to define subgroups of patients in clinical stages I and II, who have a bad prognosis because of an aggressively growing tumor (11).

Recent observations support the central role of PLKrelated kinases for the control of mitosis: A serine/threonine kinase, named Plx1, was isolated from *Xenopus* egg extracts, which exhibits close homology of 80% to human PLK, indicating that it represents its *Xenopus* counterpart. Recombinant Plx1 phosphorylated Cdc25 and stimulated its activity in a purified system, suggesting that Plx1 controls Cdc2, the cyclin dependent kinase that triggers mitosis, by regulating the activity of Cdc25 (12).

FNK/PRK expression is limited to certain tissues such as placenta, ovaries and lung (9). Re-feeding of serum-deprived cell lines of hemopoietic origin activates FNK/PRK mRNA expression. The level of FNK/PRK transcripts seems to be downregulated in lung tumors compared to uninvolved lung tissues. Murine *fnk* was shown to be an immediate early gene whose expression is first detected at 30 min after addition of various growth factors such as FGF and platelet-derived growth factor-BB to quiescent cells (8).

In a search for protein kinases, which play a role in human lung cancer, we used a PCR-based approach to amplify kinase-related sequences. Here, we report the cloning and genetic analysis of a third human PLK-related gene, which encodes a putative serine/threonine kinase closely related to murine *sak* (13). Analysis of human SAK expression in adult tissues and primary cells revealed a tissue specific expression restricted to thymus and testis.

## Materials and methods

RNA isolation and Northern blots. Tissues were homogenized in a guanidinium isothiocyanate solution (14). RNA was isolated by centrifugation through a 5.7 M CsCl cushion. For Northern blot analysis RNA was separated in a denaturing agarose/formaldehyde gel and transferred to nitrocellulose membranes (Amersham). Hybridization and washing were performed under high-stringency conditions (15). Multiple tissue blots were purchased from Clontech (Palo Alto, CA).

*PCR.* First strand cDNA synthesis and PCR were performed as described previously (16). Primers were synthesized using an Applied Biosystems 380A DNA synthesizer. Primer sequences are as follows: Eco-VHRDL (motif VHRDL), 5'-TTTGGAATTCGTNCAYMGNGAYYT-3'; Eco-P62[DEA] (motif DVWXXGM), 5'-TTGGAATTCATCCCNNNNNNC CACACATC-3'; P12(T<sup>+</sup>), 5'-GCAGAATTCGTGAACTGC GGCCGCA(dT)12-3'; EB 5'-CTGAATTCGGATCCGAACT GGTCTGACTCG-P-CH<sub>2</sub>-CHOH-CH<sub>2</sub>-NH<sub>2</sub>; EBcom 5'-CGA GTCAGACCAGTCGGATCCGAATTCAG-3', K3L2, 5'-TT CAGTCTGAGGTGTCGGGGTCTGC-3'; K3L4, 5'-GCTTCA TTTTCTGAGAAGGGTTTCAC-3'; K3U6, 5'-GTCAAAAA GAACTCTGATGCTTCTG-3'.

Labelling of probes. PCR was applied to obtain probes corresponding to aa 365-474 of SAK. Radiolabelling of the antisense strand was performed using primer K3L2 and 150  $\mu$ Ci of [ $\alpha$ -<sup>32</sup>P]dCTP (6000 Ci/mmol); 1 Ci=37 GBq.

Construction and screening of a cDNA library. Total RNA was isolated (14) from a human lung tumor (squamous cell carcinoma). Poly(A)\* RNA was selected by using oligo(dT)-cellulose (17). The construction of the cDNA library followed the method of Gubler and Hoffmann (18). In summary, a Pharmacia kit was applied for synthesis and purification of cDNA, which was ligated to EcoRI-digested  $\lambda$ gt 10 DNA. After packaging with Gigapack II Gold (Stratagene) and plating, 1.8x10<sup>6</sup> independent recombinant clones were screened under high stringency conditions (42°C, 50% formamide) with the primary PCR product as a probe derived from the catalytic domain of SAK corresponding to aa 142-184.

5' and 3' elongation by the RACE technique. Since the longest cDNA clone which was obtained by screening of the lung tumor cDNA library did not encompass the entire open reading frame of SAK, a modified RACE (rapid amplification of cDNA ends) technique was applied (19,20). For this purpose specific primers from the 5' region of the SAK-PCR product were utilized for cDNA synthesis. The cDNA was subsequently ligated to a 3' modified oligonucleotide (EB) and amplified using primer EB (com) and the SAK specific primer K3L4 resulting in the extension of 478 bp of upstream sequences. Anchored PCR was performed to complete the missing 3' portion: a cDNA starting at the poly A-tail was synthesized with P12(T<sup>+</sup>). Subsequent PCR amplification with the specific primer K3U6 located at the 3' end of SAK and primer P12(T) provided the complete open reading frame.

Sequencing. Sequencing of double-stranded DNA was performed by the dideoxy chain termination method with

Taq polymerase using an ABI 373A DNA sequencer according to the protocols of the manufacturer (Applied Biosystems, Weiterstadt). Cycle sequencing on a DNA thermal cycler (Perkin Elmer Cetus) was performed with dye terminators.

## Results

PCR-based isolation of a PLK related protein kinase cDNA from human tissues. cDNA from human embryonic tissues was amplified using primers corresponding to highly conserved amino acid motifs (VHRDL and DVWXXGM) from subdomains VI and IX within the catalytic domain of protein kinases (21) in order to identify novel members of the kinase family and to evaluate their role in cancer development. Resulting PCR products were ligated to the pBluescript KS(\*) vector (Stratagene). Sequence determination of 280 inserts revealed various unknown sequences related to protein kinases: Specific extension of two unknown kinase related sequences yielded the complete open reading frames of the new protein-kinases PLK (7) and MO15 (22). Since this approach has been shown to provide new kinases which are of importance for lung tumors such as PLK, a marker for cellular proliferation with prognostic significance for NSCLCpatients, we have chosen an additional clone, named K3, representing a sequence related to PLK for further studies. A 200 bp-insert derived from K3 was used as a probe for the screening of a cDNA library based on RNA from a human lung tumor (squamous cell carcinoma). We obtained a clone of 1.3 kb. The RACE technique was applied for 5' and 3' elongation of this cDNA in order to obtain the complete open reading frame. The analysis of the elongated clone revealed a continuous sequence of 3.1 kb with a complete open reading frame exhibiting an ATG and stop codon. Using cDNA from normal lung tissues we verified the sequence of 3.1 kb by PCR-amplification and direct sequencing.

Identification of a human serine/threonine kinase gene related to the mouse gene sak. Determination of the nucleotide sequence exhibited a single open reading frame of 2910 nt extending from an ATG codon at position 141 to an in-frame stop codon at position 3051, which predicted a 109kDa polypeptide of 970 aa (Fig. 1). The 5' untranslated sequence has a length of 140 bp with a putative start codon which is in agreement with Kozak's rule for the initiation of translation (23). The 3' untranslated sequence is 39 bp in length containing a potential polyadenylation signal (AATAAA). The predicted K3-polypeptide contains an aminoterminal kinase domain which shows the characteristics of protein serine/threonine kinases. Interestingly, a lysine residue located within the motif HRDLK, which is conserved in almost all members of this family, is substituted by a threonine residue (aa 138) in the putative K3-amino acid sequence. A computer analytic comparison of the 970 aapolypeptide with other known proteins (Swiss-Prot and the Protein Identification Resource, September 1996) confirmed the first observation that it belongs to the family of serine/threonine kinases. In particular it is homologous to polo-related kinases: Comparing the kinase domains of the new protein to those of human PLK, human FNK/PRK and

PTTCA005T03C030C03C010C0A005C03C02C02C02C02C02C02C02C02C02C02C02C02C02C	20 140
ATGCCACCTGCATCGGGGGGAGAGAGATCGAAGGTGGGAGAAGCTGCTTAUGTAAGGATCATTTCCTKKTTGCTKATGCATCCATTCACACGGGTTTGCAAGTTGCAATC R A T C I G E K I E D P X V S N L L G K G S F A G V Y R A E S I N T G L E V A I	260
	380 80
CATROCALTERTOTOTATE CALARGENCE CATAFORNOLIA ANDRA A DOTATE TAAGAATGAACOUTTOTE MAAAA TAAAGAATGAACTE CAUATE D S M V V Y L V L B N C R B S B M R T L K N S V K P F S B N B A R B F M H Q I	500 120
	620 160
	740 200
	860 240
	980 282
GACTEANTREATASTUGUEATUCAEAATTUTACTUCAATTACAGETTETTECAGTACEAGTACEAGTAGTAGTAGTAGTAGAAGAAGAETTTEGATTOGTEAGEGAETGECAAAT 1 D S T D S G H A T I S T A I T A S S G T S I S G S L P D K R R L L I G O P L P R	100 320
AAAATGACTGTATTICCAAGGAATAAAAGTTCAACTGATTITICTTCAGGAGAATGGAAACAGTYTTTATACTCAGTGGGGAAATCAAGAAACCAGTAATAGTGGAAGAGTA K N T V F P K N K S S T D F S S S G D G N S F Y T O N G N O E T S N S G R G R V	220 362
ATTCAAGATOCAGAAGAAAGGCCACATTCTCGATACCTTCGTAGAGCTTATTCCTCTGATAGAACGACTCTAATAGTCAGTC	340 400
NCAGCAGAAATGCTTTCAUTGTCAAAAAATGAGGAGGAGGTGAAAAATGAAGAGGAGTACTGCGCACAGAACAATGCCAACAATGCCATTTCAUTGTAAGGACGAGAAAGACATCAGTGAAGAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGA	460 440
ICTOGATCTITTGAAAGACCTGATAACAATCAAGCACTCTCCAATCACCACTTTGCCATTGCAATTGCAATCGCCGACACCTCAGACTGAAACCGTACAACAGTGGTFT 1 8 G S F E R P D N N Q A L S N H L C P G R T P F P F A D F T P O T E T V Q Q K F	380 490
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БАТЖСАЛАВОТСАЛЛАЛСЯСТВАТИСТОТИТАТАТССАЛТТСТОТАЛЛАГАКСАЛЛАТАССАТОЛЛАГТАТАТАТСКСТОСАСТВОСАТАОТАЛААСТОЛЛАТАТССАЛСАЛДАЛТОТ Ц D T K V E K N S D A S D N A H S V K O Q N T M K Y M T A L H S K P E 1 I Q Q K E	820 560
UTTIFUGCTURGATUCTCTTTCTURGAGGARGACTARGGGGTATUGGGCGTATUGGGCTATGAGATTATAGAACATTACATCTCUTTTCTURGACCATACATCTACATCTCUTTAGAGGGGTAAAAA L V P G S D P L S E Q S K T B G K E P P N G Y Q N B T L B S I T S P L V A N B L R	940 600
CCARCEGRE CRAARACCERARACOCTOROGTOROGTORECETEREREGENOTOTOTOGRECTUTERAGENOTATOTEREGRETETURGENETTOTURGENETTOTTURGENETTOTTURGENETTOTURGENE PIKOVT KKAVVSILDE KKVCVELUVKEVASOT	068 649
NOTINATIONALATACUATCACTATTTATTATCAAAROOTUGTAGAUTTTTCCTUTNICTUATAGACCACUCTURICTACIACAACATCAUPAUCACACEPPUACAAPPACUAGAA 2 S D G N T L T L Y P N G G R G P P L A D R P P S P T O N I S F T S F D N L P F I	180
илтастоводлалатательна вестгосадоттаталарстваларствалаленского салалатателе стала посталато статятовадаате тестоват и К Y K R K Y Q T A S R F V Q L V R S K S P K I T Y F T K Y A K C I L K B N S F G	300
ICTURTITTGROFFINGTFTTRATGROGGTARARATRCRCRARGROADGATTCATCACUTURTCRCRCRCRCRCRCRCRCRCPLALACTTRALLAGTGARGTGARGTGARGTANTACC 2 A D F E V N F Y D G V K I H K T E D F I Q V I E K T G K S Y T L K S S S V N S	420 760
TTUAAAUAGGAGATAAAAATUTTTATUGACGATUCTAATGAGGEGATGGTATUGTTTUTTTUGAGGGAAAGTAGAAAGGAAAAGGAAAAGGAAAAGGAAAACTAGGAGGGCCCCTTTTUCCG 2 L K E E I K M F N D N A N E G H R I C L A L E S I T S K K K K K K K S A F F F F 2	540 800
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CAGGAGTGTCTTCTATCAGTTATACGTUACGAAATGGTCAAAGAGTAGGTATGGAGAAAATGGAGAAAATTACCAGACATCAAACAGAAATTACAGTGTCTGTC	1
NGFFTTCTRATCCGRCTCCTARTTTCAT <u>TCA</u> TTRARRCTCCTTTCAGRCATATINGTT <b>TATTRAT</b> INGCT	091 976
	urdar.

Figure 1. Nucleotide and amino acid sequence of human SAK (K3) including partial untranslated sequences. The start codon is in bold type. The stop codon is underlined. The highly conserved motifs of the kinase domain (shaded) 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* (21,26).

murine snk (24) the similarity was determined to be 37%, 41% and 39% respectively. The closest overall homology of 82.3% has been detected to a murine gene, which was named sak (13). Comparing the kinase domain and the carboxyterminal portions of K3 and sak we observed similarities of 94.4% and 77.2%. The relationship of both genes (K3/sak) was confirmed by the homologies of 62% (140 bp) and 66% (40 bp) within the 5' and 3' untranslated regions. K3 exhibits the same topology as the members of the polo-family (polo, PLK, FNK and snk) with the kinase domain located at the aminoterminal portion of the protein. In contrast to these proteins K3 does not contain the polohomology 2 (PH2) domain, which is a motif of approximately 30 aa within the carboxyterminal region of polo-related kinases. Due the high degree of homology it seems likely that the 910 aa-polypeptide is the human counterpart of the murine gene sak (Fig. 2). Thus, we suggest to name it also SAK.

Fig. 3 shows the evolutionary relationship of the kinase domain of SAK to the rest of human serine/threonine kinases. In a phylogenetic tree based on sequence homologies within the kinase domain the split of SAK occurred before the evolution of the family of polo related kinases.

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SAK exhibits tissue specific expression. The Northern blot analysis of RNAs isolated from various human tissues with a specific probe from the carboxyterminal portion of the open reading frame showed SAK transcripts of 4.0 kb to be most abundant in testis and thymus (Fig. 4). In all other tissues examined SAK mRNA was below the limit of detection under high stringency conditions. Furthermore, we carried out a Northern blot analysis of RNAs isolated from a variety of different human malignant tissues. Human tumors of various origin such as lung, breast and brain and corresponding normal tissues from the same patient did not exhibit SAK mRNA expression (data not shown). 508

K3 Sak	MATCIGEKIEDFKVGNLLGKGSFAGVYRAESIHTGLEVAIKMIDKKAMYK	50
	····	
K3 Sak	AGMVQRVKNEVKIHCQLKHPBILELYNYPEDSNYVYLVLEMCHNGEMNRY	100
K3	LENRVEPFGENEARHFMHOITTGMLYLHSHGILHRDLTLSNILL TENMINT	
Sak	LANKVAPPSENEARHIMHQIITGMIYLHSHGILHRDLTLSNLLLTHNMNI 	150
K3	KIADFGLATQLKMPHEKHYTLOGTPNYISPEIATRSAHGLESDVWSLGCM	200
Sak	II	200
КЗ	FYTLLIGRPPFDTDTVKNTLNKVVLADYEMPTFLSIEAKDLIHQLLRRNP	250
Sak	S	200
КЗ	ADRLSLSSVLDHPFMSRNSSTKSKDLGTVEDSIDSGHATISTALTASSST	300
Sak	·····.MLTG.	
K3	SISGSLFDKRRLLIGQPLPNKMTVFPKNKSSTDFSSSCDGNSPYTQWGN-	349
Sak	.LLVIQN.SSN.CP	
K3 Sak	-QETSNSGRGRVIQDAEERPHSRYLRRAYSSDRSGTSNSQSQAKTYTMER EANSREHASPRSV.	398
K3 Sak	CHSAEMLSVSKRSGGGENEERYSPTDNNANIFNFFKEKTSSSSGSFERPD	448
K3		
Sak	NNQALSNHLCPGKTPFPFADPTPQTETVQQWFGNLQINAHLRKTTEYDSI E.HSH.LQM.MMGE.N.HHTV	498
K3 Sak	SPNRDFQGHPDLQKDTSKNAWTDTKVKKNSDASDNAHSVKQQNTMKYMTA	548
K3 Sak	LHSKPEIIQQECVFGSDPLSEQSKTRGMEPPWGYQNRTLRSITSPLVAHR H.HVMPPLH.HN.SSTLKPTI	598
КЗ	LKPIRQKTKKAVVSILDSEEVCVELVKEYASQEYVKEVLQISSDGNTITI	648
Sak		
КЗ	YYPNGGRGFPLADRPPSPTDNISRYSFDNLPEKYWRKYQYASRFVQLVRS	698
Sak	DLI	
K3 Sak	KSPKITYFTRYAKCILMENSPGADFEVWFYDGVKIHKTEDFIQVIEKTCK .T	748
K3 Sak	SYTLKSESEVNSLKEEIKMFMDHANEGHRICLALESIISEERKTRSAPF NN.N.TV.VYSVKRS.GSS.	798
K3 Sak	FPIIIGRKPGSTSSPKALSPPFSVDSNYPTRDRASPNRMVMHSAASPTQA VNAPSCCKGEQ.AS.LSVNFS	848
K3 Sak	PILNPSMVTNEGLGLTTTASGTDISSNSLKDCLPKSAQLLKSVFVKNVGW .G.S.T.VH.A.T.GV.S	898
кз	ATOLTSGAVWVOFNDGSOLVVOAGVSSISYTSPNG0TTRYGENEKI, PDV1	948
Sak		
K3	KOKLOCLSSILLMFSNPTPNPH	970
Sak	Q	

Figure 2. Sequence alignment of the predicted human SAK (K3) and murine *sak* amino acid sequences. Numbers to the right refer to the last amino acid in this line. Identical residues in the murine sequence are substituted by dots. Gaps represented by dashes were inserted to maximize the alignment. The shaded region represent the kinase domain.

#### Discussion

In this study we identified a novel member of the family of serine/threonine kinases which is related to the *Drosophila melanogaster* gene polo. We describe the cloning of the new gene during a screen of a cDNA library based on human RNA designated to isolate new protein kinase genes which might participate in the development of lung cancer. The closest relationship of 82.3% has been found to murine sak. Maximal alignment required the introduction of several gaps (Fig. 2). Within the family of kinases required for the

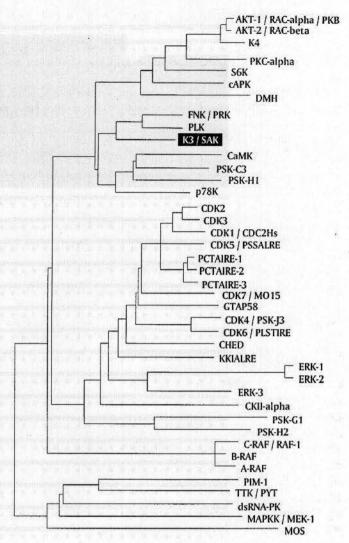


Figure 3. Phylogenetic relationship of SAK to other human serine/threonine kinases. The amino acid sequences of the catalytic domains of human serine/threonine kinases were used for calculation of a phylogenetic tree with the Tree program of HUSAR (Heidelberg Unix Sequence Analysis Resource, DKFZ, Heidelberg). It is based on the progressive alignment method of Feng and Doolittle (27) in a multiple sequence alignment.

progression through the cell cycle we compared the open reading frames of human/murine counterparts and determined the homology to be 93.7% for PLK/plk, 91.8% for FNK/fnk and 95.1% and human/murine MO15. This comparison revealed that SAK is not as well conserved as other members of the polo-family. Still, the homologies of

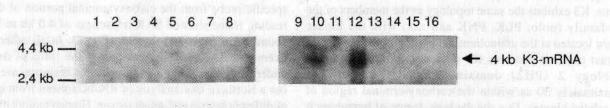


Figure 4. Expression of SAK (K3) mRNA in adult tissues. Each lane contained 2  $\mu$ g of poly(A)<sup>+</sup> from human heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas (lanes 1-8) as well as spleen, thymus, prostate, testis, ovary, small intestine, colon and peripheral blood leukocytes (lane 9-16). Hybridization was done under high stringency with an antisense probe corresponding to aa 365-474. Size markers on the left are in kilobases.

An analysis of SAK expression by Northern blot hybridization has shown that out of 16 adult tissues SAK transcripts are restricted to testis and thymus. A detailed study of various tumor tissues and adjacent normal tissues from the same patient revealed that human SAK transcripts are not detectable in those specimens. These data indicate that human SAK is not expressed in the proliferative active tissues examined. This observation differs in some way from a study of sak expression in murine tissues which has shown that in the embryonic central nervous system sak transcripts are restricted to the ventricular zones, where neuroblasts are dividing. Murine sak transcripts are not detected in zones, where the postmitotic neurons are located (13). In adult mice, sak is expressed in tissues with a mitotic component, including hemopoietic tissues and the stem cells of the intestinal crypt. Furthermore, high levels of murine sak mRNA were found in meiotic spermatocytes and oocytes (13).

Expression of human SAK differs also from PLK, which belongs to the family of polo-related kinases. PLK was shown to be regulated during the cell cycle in NIH3T3 cells (4). In addition, PLK mRNA is highly expressed in rapidly dividing cell populations found in fetal and newborn tissues and adult hemopoietic tissues (25). Therefore, PLK mRNA expression is strongly correlated with the mitotic activity of cells and tissues. In our own studies on the function of PLK most human tumors of various origins were found to express high levels of PLK mRNA and protein, although its expression was not detectable in normal tissues, indicating that the expression of PLK is associated with cell proliferation (7,10,11).

In addition to the diverging pattern of mRNA expression the architecture of the putative SAK protein differs clearly from the related human polypeptides PLK and FNK/PRK: Close relatives of *Drosophila melanogaster polo* such as *Saccharomyces cerevisiae* CDC5 and human PLK, FNK/PRK exhibit a common domain, named polohomology-(PH2) domain, with an unknown function. SAK lacks this motif of polo-related kinases. Thus, despite different structural similarities of SAK and polo-related kinases, the tissue distribution patterns of their mRNAs as well as the molecular design of the putative proteins are distinct, suggesting that these kinases have special physiological roles in different cells.

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## References

- Llamazares S, Moreira A, Tavares A, Girdham C, Spruce BA, Gonzalez C, Karess RE, Glover DM and Sunkel CE: Polo encodes a protein kinase homolog required for mitosis in *Drosophila*. Gene Develop 5: 2153-2165, 1991.
- Drosophila. Gene Develop 5: 2153-2165, 1991.
  Kitada K, Johnson AL, Johnston LH and Sugino A: A multicopy suppressor gene of the Saccharomyces cerevisiae GI cell cycle mutant gene dbf4 encodes a protein kinase and is identified as CDC5. Mol Cell Biol 13: 4445-4457, 1993.
- Sunkel CE and Glover DM: Polo, a mitotic mutant of Drosophila displays abnormal spindle poles. J Cell Sci 89: 25-38, 1988.
- Lake RJ and Jelinek WR: Cell cycle and terminal differentiationassociated regulation of the mouse mRNA encoding a conserved mitotic protein kinase. Mol Cell Biol 13: 7793-7801, 1993.
- Golsteyn RM, Schultz S, Bartek J, Ziemiecki A, Ried T and Nigg EA: Cell cycle analysis and chromosomal localization of human Plk1, a putative homologue of the mitotic kinases *Drosophila polo* and *Saccharomyces cerevisiae* Cdc5. J Cell Sci 107: 1509-1517, 1994.
- 6. Hamanaka R, Maloid S, Smith MR, O'Connell C, Longo DL and Ferris DK: Cloning and characterization of human and murine homologues of the *Drosophila polo* serine-threonine kinase. Cell Growth Differ 5: 249-257, 1994.
- Holtrich U, Wolf G, Bräuninger A, Karn T, Böhme B, Rübsamen-Waigmann H and Strebhardt K: Induction and downregulation of human PLK, a serine/threonine kinase expressed in proliferating cells and tumors. Proc Natl Acad Sci USA 91: 1736-1740, 1994.
- Donohuc PJ, Alberts GF, Guo Y and Winkles JA: Identification by targeted differential display of an immediate early gene encoding a putative serine/threonine kinase. J Biol Chem 270: 10351-10357, 1995.
- Li B, Ouyang B, Pan H, Reissmann PT, Slamon DJ, Arceci R, Lu L and Dai W: Prk, a cytokine-inducible human protein serine/threonine kinase whose expression appears to be downregulated in lung carcinomas. J Biol Chem 271: 19042-19408, 1996.
- Yuan J, Hörlin A, Stutte HJ, Rübsamen-Waigmann H and Strebhardt K: Polo-like kinase, a novel marker for cellular proliferation. Am J Pathol (In press).
- Wolf G, Elez R, Doermer A, Holtrich U, Ackermann H, Stutte HJ, Altmannsberger H-M, Rübsamen-Waigmann H and Strebhardt K: Prognostic significance of polo-like kinase (PLK) expression in non-small cell lung cancer. Oncogene (In press).
- Kumagai A and Dunphy WG: Purification and molecular cloning of Plx1, a Cdc25-regulatory kinase from *Xenopus* egg extracts. Science 273: 1377-1380, 1996.
- Fode C, Motro B, Yousefi S, Heffernan M and Dennis JW: Sak, a murine protein-serine/threonine kinase that is related to the *Drosophila polo* kinase and involved in cell proliferation. Proc Natl Acad Sci USA 91: 6388-6392, 1994.
- Chirgwin JM, Przybyla AE, MacDonald RJ and Rutter WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18: 5294-5299, 1979.
- Sambrook J, Fritsch EF and Maniatis T: Molecular cloning: a laboratory manual. 2nd edition. Cold Spring Harbor Laboratory Press, Plainview, NY, 1989.
- 16. 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.
- Aviv P and Leder P: Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acidcellulose. Proc Natl Acad Sci USA 65: 1408-1412, 1972.
- Gubler U and Hoffmann BJ: A simple and very efficient method for generating cDNA libraries. Gene 25: 263-269, 1983.
- Böhme B, Holtrich U, Wolf G, Luzius H, Grzeschik K-H, Strebhardt K and Rübsamen-Waigmann H: PCR mediated detection of a new human receptor-tyrosine-kinase, HEK 2. Oncogene 8: 2857-2862, 1993.
- 20. 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.

- 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.
- Kobelt D, Karn T, Hock B, Holtrich U, Bräuninger A, Wolf G, Strebhardt K and Rübsamen-Waigmann H: Human and Xenopus MO15 mRNA are highly conserved but show different patterns of expression in adult tissues. Oncol Rep 1: 1269-1275, 1994.
- Kozak M: An analysis of vertebrate mRNA sequences: intimations of translational control. (Review) J Cell Biol 115: 887-903, 1991.
- Simmons DL, Neel BG, Stevens R, Evett G and Erikson RL: Identification of an early-growth-response gene encoding a novel putative protein kinase. Mol Cell Biol 12: 4164-4169, 1992.
- 25. Clay FJ, McEwen SJ, Bertoncello I, Wilks AF and Dunn AR: Identification and cloning of a protein kinase-encoding mouse gene, Plk, related to the polo gene of *Drosophila*. Proc Natl Acad Sci USA 90: 4882-4886, 1993.
- 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.
- Feng D-F and Doolittle RF: Progressive sequence alignment as a prerequisite to correct phylogenetic trees. J Mol Evol 25: 351-379, 1987.
- the better galaxies of homeon PLE, a heritarithe reaction binary extremend in positionalise collected transfer. Provide the  $X_{10}$  and  $V_{20} = 0$ , and  $V_{20} = 0.000$ . If the reaction PLE have the Court Y and Window De International by targeted differences in evolve of an international court genes by targeted differences in evolve of an international court genes by targeted differences in evolve of an international court of the evolve of an international construction of an international court of the second courter of the evolve of an international court of the evolve of the evolve of an international court of the evolve of the evolv
- Lu S. Donneg R. Franki, Restaurant P. Showan D. Arabal K. Lu T. and Dia W. Pro, a generative instantish futuring generative restaution states in the states in a second structure of the state restaution in here consistence. J Mat Cases 211 (2012-1986).
- 10. Yantu J. Markin A. Sawtor HI. Waterman Weignedge Heart Structures for Pate-take televase, a recent marker for califold and force berg day J. Patert Society Sciences.
- Will G. Black Deen new Achineme W. Actornment F. Sente H. Alikouescherger MAC, ethly news: Waterstan R and Arthour th R. Programics respectives at pole-tills Alexan (PUA) experiment. In new world cell tanks measure. University Internet.
- Kamagai A and Daminy Wei? Participton and momentidisade of PRU a City25 regulatory kinete from Jone of g extenses. Sense: 178 15975 (10), 1996.
- 13. Fork C. Maran B. Young X. McDonn M. and Daron. N. 1985. Control of the second state of the second se
- (4) Chargesta gib. Prophytica AE, MacDonald KJ and Kester KJ, handston of minispatify active effectiveless and free-manages. application for interpretation. Historycanomy 18, 1214, 1219, 1219.
- Sambonski, Prakob EP and Matchine T. Minkradar President Interalisty apprend. Text produce Could Spring Hactor Enforctiony Press, Humanow, NY, 1985.
- constant of the A structure is requirement. A deviate of a location of constant constant parameter for a structure of order devices and the structure of the structure structure of the stru
- Solution manufile even the strain of the manufield has I service a
- Barban A., Mednich D., Weit C., Galess H. Sternmitz E.H. Formhundt K. gen Willington Willingtons N. N.K. and det Microsoft et a cast function endpriced pressness function. J Net Co. National Information Press, 2018.
- Amir T. Mainten, G. Somerner, A. Bohne, R. W. eff, G. Santar, K. W. eff, G. Santar, K. W. eff, G. Santar, S. Santar, Sant

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Equestion of human EAH differentiated theore PLK, when belongs to the family of pole-related theorem PLK was donver to be regulated during the cult cycle in NIETT cells (0) in addition. PLK wiRNA is bightly expressed in rapidly dividing cell populations band in fittl and revision theorem and with homopoletic statics (25). Therefore, PLK mERA code and traces for our own materi as due found or represtate torung to not own materi as due found or represtate torung torungs of suffere wights were found or represtate torung torungs of suffere wights were found or represtate torung torungs of suffere wights were found or represtate torung torungs of suffere wights were found or represtate torung torungs of suffere wights were found or represtate torung torungs of suffere wights were found or represtate torung torungs of suffere wights were found or represtate torung torungs of suffere wights were found or represtate torung torungs of suffere wights were found or represtate torung torungs of suffere wights with only providentian expression of PLK to antoremated with only providentian (20,0,11).

In oridition to the divergence pattern of mRMA expression the architecture of the patterns SAR present differs clearly draw the related bomon polypeptides PLX and PRADPRS. Clear relatives of Drompidla and anopative with ranks in Section correspond to an interceptive with ranks PLAC PRADPRS exhibit a common domain, named pellobaratory (PriD) domain, with an anterware function SAR access the most of protocols of sack and pelloted errors structural function related to an externation of the relation of protocols of sack and pelloted errors the angle of protocols of sack and pelloted errors the angle of protocols patterns of the related to an externation of protocols patterns of the related performing the theory direction protocols protocols and any and another to be protocols and pello-tracted to any and a structural function of the patterns of the relative performs the theory binance investigation of the patterns of the regiment of the patterns of the patterns of the relative and the relative binance investigation of the relative of the relative and the relative binance investigation of the relative of the relative and the relative binance investigation of the relative of the relative to the relative and the relative binance investigation of the relative of the re

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