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# Induction and down-regulation of PLK, a human serine/threonine kinase expressed in proliferating cells and tumors

(signal transduction/proliferation-associated kinase/mitogen induction/lymphocytes)

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ABSTRACT **We have identified the nucleotide sequence of the cDNA encoding the human counterpart of the mouse gene *Plk* (polo-like kinase). The sequence of the human gene, *PLK*, predicts a serine/threonine kinase of 603 aa. Expression of *PLK* mRNA appeared to be strongly correlated with the mitotic activity of cells. Resting peripheral lymphocytes did not express the gene at all. When primary T cells were activated by phytohemagglutinin, a high level of *PLK* transcripts resulted within 2–3 days. In some cases, addition of interleukin 2 to these cells increased the expression of *PLK* mRNA further. In contrast, primary cultures of human peripheral macrophages, which were not dividing under the culture conditions applied, showed very little or no *PLK* mRNA. Stimulation of these cells by bacterial lipopolysaccharide, an inducer of several cytokines in macrophages, totally abrogated the expression of *PLK* mRNA. In line with a function of *PLK* mRNA expression in mitotically active cells is our finding that six immortalized cell lines examined expressed the gene. In A-431 epidermoid carcinoma cells this expression was down-regulated by serum starvation and enhanced after serum was added again. Tumors of various origin (lung, colon, stomach, smooth muscle, and esophagus as well as non-Hodgkin lymphomas) expressed high levels of *PLK* transcripts in about 80% of the samples studied, whereas *PLK* mRNA was absent in surrounding tissue, except for colon. The only normal tissues where *PLK* mRNA expression was observed were colon and placenta, both known to be mitotically active. No *PLK* transcripts were found in normal adult lung, brain, heart, liver, kidney, skeletal muscle, and pancreas. In Northern blot experiments with RNA from lymphocytes which were treated with phytohemagglutinin and cycloheximide, *PLK* transcripts were not detectable, suggesting that *PLK* is not an early growth-response gene.**

The signaling network for the regulation of cell proliferation is extremely complex and controlled by various mechanisms, most of which are based on phosphorylation of proteins at their tyrosine, serine, and/or threonine residues. One pathway for the transduction of signals from the cellular environment to the cytoplasm is initiated by the binding of growth factors to receptor tyrosine kinases at the cell surface. For example, binding of epidermal growth factor (EGF) to its receptor (EGFR) induces dimerization and subsequent autophosphorylation of the carboxyl terminus of EGFR (1–3). A cytoplasmic complex of Grb2 and Sos proteins associating via two Src homology 3 (SH3) domains of Grb2 and proline-rich regions of Sos, binds with the SH2 domain of Grb2 to autophosphorylated tyrosines of the activated EGFR. By this association, Sos is translocated into the proximity of the membrane-bound Ras protein and activates Ras by releasing GDP (4–8). Activated Ras mediates signal transmission from receptor tyrosine kinases to a cascade of serine/threonine kinases including c-Raf, MEK, MAP kinase, and RSK (9). Many observations suggest that MAP kinase and RSK, which are located in the cytoplasm and in the nucleus, directly influence gene expression by phosphorylation of transcription factors (10, 11). Very recently, a MEK kinase independent of Raf-1 has been described (12). Still, many components of this mitogenic cascade, such as protein kinases and many of their interactions, remain to be discovered.

We and others have cloned cDNAs of novel protein kinases by using degenerate oligonucleotide primers for the PCR amplification of reverse-transcribed mRNA (13–17). A principal attraction of this approach is that based on conserved motifs of the kinase domain it allows amplification of protein kinases which are expressed at very low frequency. Here, we describe the PCR-based identification of a gene coding for a protein kinase from embryonic tissue. The complete cDNA was subsequently isolated from a cDNA library based on RNA from a squamous-cell lung carcinoma. The cDNA encodes a protein which seems to be the human counterpart of a mouse protein referred to as Plk (polo-like kinase) and contains structural hallmarks of protein-serine/threonine kinases.† We show that expression of human *PLK* mRNA is increased in proliferating tissues like human tumors, as well as in cell lines and growth-stimulated primary cells. In quiescent cells, *PLK* transcripts were not found. These data suggest that *PLK* mRNA expression is tightly linked to proliferation.

## MATERIALS AND METHODS

**Tissue Samples.** Tissue samples were obtained from 72 patients undergoing surgical resection at the Nordwest Hospital in Frankfurt. Whenever possible, surrounding normal tissue was also obtained. The samples were stored at −70°C.

**Cell Culture.** Human lymphocytes and macrophages from peripheral blood were cultivated as described (18). For cycloheximide treatment, T lymphocytes were stimulated for 1 day with phytohemagglutinin (PHA). Subsequently, cycloheximide was added (75 μM) and RNA was isolated after 24 hr to study the influence of protein synthesis on the induction of *PLK*.

**DNA Isolation and Southern Blots.** DNA was prepared as described (19). For Southern blot analysis, 10-μg aliquots of DNA were digested with restriction endonucleases (New England Biolabs), electrophoresed in a 1% agarose gel, and transferred to nylon membranes (Amersham) (20).

**RNA Isolation and Northern Blots.** Tissues were homogenized in a guanidinium isothiocyanate solution (21). RNA was

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Abbreviations: IL-2, interleukin 2; NLS, nuclear localization signal; PHA, phytohemagglutinin.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. X75932).

isolated by centrifugation through a 5.7 M CsCl cushion. For Northern blot analysis, RNA was electrophoresed in a denaturing agarose/formaldehyde gel and transferred to nitrocellulose membranes (Amersham). Hybridization and washing were performed under high-stringency conditions (20, 21).

**PCR.** First-strand cDNA synthesis and PCR were performed as described (13). Primers were synthesized on an Applied Biosystems 380A DNA synthesizer. Primer sequences are as follows: ECO-P6[2DEA], 5′-TTGGAATTCATCCCNNNNNNCCACACATC-3′; 17low, 5′-TGATGTTGGCACCTGCCTTCAGC-3′; ECO-VHRDL, 5′-TTTGGAATTCGTNCAYMGNGAYYT-3′.

**Labeling of Probes.** PCR was used to obtain a probe corresponding to aa 285–497. Radiolabeling of the antisense strand was performed using primer 17low and 150 μCi of [α-$^{32}$P]dCTP (6000 Ci/mmol; 1 Ci = 37 GBq).

**Construction and Screening of a cDNA Library.** Total RNA was isolated (21) from a human lung tumor. Poly(A)$^+$ RNA was selected by using oligo(dT)-cellulose (22). The construction of the cDNA library followed the method of Gubler and Hoffmann (23). In brief, a Pharmacia kit was used for synthesis and purification of cDNA, which was ligated to *Eco*RI-digested λgt10 DNA. After packaging with Gigapack II Gold (Stratagene) and plating, 1.8 × 10$^6$ independent recombinant plaques were screened under high-stringency conditions (42°C, 50% formamide) with a probe derived from the catalytic domain corresponding to aa 174–238.

## RESULTS

**PCR-Based Isolation of a Protein Kinase cDNA from a Human Lung Tumor.** cDNA from human embryonic tissues was amplified by using primers (ECO-VHRDL and ECO-P6[2DEA]) corresponding to the highly conserved amino acid motifs from subdomains VI and IX (VHRDL and DVWXXG, respectively) of protein kinases (24). The PCR products were ligated to the pBluescript KS(+) vector (Stratagene). Sequence determination of 280 clones revealed 7 unknown putative protein kinases. One of these clones was highly related to Raf-1 and polo. This clone was used as probe to screen 1.8 × 10$^6$ recombinant clones from an oligo(dT)-primed cDNA library in λgt10 based on poly(A)$^+$ RNA from a human lung tumor. Six positive clones ranging from 1.5 to 2.1 kb were obtained.

**Identification of a Human Serine/Threonine Kinase Gene Closely Related to the Mouse Gene *Plk*.** Determination of the nucleotide sequence of the longest clone, designated Lambda 4, resulted in a sequence of 2136 bp with a single open reading frame of 1809 nt extending from an ATG codon at position 72 to an in-frame stop codon at position 1881, predicting a 68-kDa polypeptide of 603 aa (Fig. 1). The sequence surrounding the potential start codon is in agreement with Kozak's rule (25), suggesting that it is used for the initiation of translation. A computer-aided comparison of the 603-aa sequence with other published sequences (Swiss-Prot and the Protein Identification Resource, February 1993) showed that the protein is related to members of the serine/threonine kinase family. Most prominent is the 93% homology to a gene which has been isolated recently from a primitive murine hemopoietic progenitor cell line and which was named *Plk* (polo-like kinase) due to its close relationship to the mitosis-associated protein kinase polo from *Drosophila melanogaster* (26–28). Most amino acid substitutions between Plk and Lambda 4 are located in the extreme amino-terminal portion of the proteins. Due to the high degree of homology and the same domain topology, it seems likely that Lambda 4 represents the human homologue of the mouse gene *Plk*. Therefore, we propose to call it *PLK*.

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MSAAVTAGKLARAPADPGKAGVPGVAAPGAPAAAPPAKEIPEVLVDPRSRRRYVRGRFLG  60
KGGFAKCFEISDADTKEVFAGKIVPKSLLLKPHQREKMSMEISIHRSLAHQHVVGFHGFF 120
EDTDFVFVVLELCRRRSLLEPHKRRKALTEPEARYYLRQIVLGCQYLHRNRVIHRDLKLG 180
NLSLNEDLEVKIGDFGLATKVEYDGERKKTLCGTPNYIAPEVLSKKEHSFEVDVWSIGCI 240
MYTLLVGKPPFETSCLKETYLRIKKNEYSIPKHINPVAASLIQKMLQTDPTARPTINELL 300
NDEFFTSGYIPARLPITCLTIPPRFSIAPSSLDPSNRKPLTVLNKGLENPLPERPREKEE 360
PVVRETGEVVDCHLSDMLQQLHSVNASKPSERGLVRQEEAEDPACIPIFWVSKWVDYSDK 420
YGLGYQLCDNSVGVLFNDSTRLILYNDGDSLQYIERDGTESYLTVSSHPNSLMKKITLLK 480
YFRNYMSEHLLKAGANITPREGDELARLPYLRTWFRTRSAIILHLSNGSVQINFFQDHTK 540
LILCPLMAAVTYIDEKRDFRTYRLSLLEEYGCCKELASRLRYARTMVDKLLSSRSASNRL 600
KAS                                                             603
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FIG. 1. Amino acid sequence of human PLK. The deduced amino acid sequence of PLK is given in one letter code. Numbers at right indicate positions of amino acids. Motifs for the initial PCR amplification are underlined. The boundaries of the putative kinase domain are marked by bent arrows. Amino acids of the motif involved in ATP binding are boxed. Clusters of basic amino acids, assumed to be nuclear localization signals, are shaded.

The amino-terminal portion of the putative protein PLK contains highly conserved sequence motifs of serine/threonine kinases: According to Hanks *et al.* (24) the catalytic domain is divided into 11 subdomains. The consensus sequence GXGXXG (subdomain I) found in many nucleotide-binding proteins and in kinases is modified in PLK as GXGXXA motif. This ATP-binding site of PLK, however, is in common with the corresponding sequence of its murine counterpart (26) and related serine/threonine kinases such as polo (28), Snk (29), and MSD2, a *Saccharomyces cerevisiae* cell cycle kinase (26), as well as Kin-28 (24). The following motifs in subdomains VI (HRDLKLG, aa 174–180) and VIII (GTPNYIVPE, aa 213–221), which are characteristic for a number of serine/threonine kinases, are almost identical between the murine and human homologues of PLK.

In addition to the motifs characteristic for protein serine/threonine kinases, we identified various short basic amino acid sequences in the amino-terminal half of the protein: RRR (aa 50–52 and 134–136), KRRK (aa 143–146), and RKK (aa 207–209) (Fig. 1). Such short stretches of basic amino acids, referred to as nuclear localization signal (NLS) sequences, are a common feature of proteins which accumulate in the cellular nucleus (30, 31).

***PLK* mRNA Is Highly Expressed in Cells and Tissues with Proliferative Activity.** We carried out Northern blot analysis of RNAs isolated from a variety of different human normal and malignant tissues and various cell lines to examine the expression of *PLK*. *PLK* transcripts of 2.3 kb were detected in placenta, but in adult heart, brain, lung, liver, skeletal muscle, kidney, and pancreas, *PLK* mRNA was at or below the limit of detection (Fig. 2). An expanded screening of human tissues, including esophagus, colon, stomach, and spleen, revealed *PLK* mRNA only in colon (data not shown). In contrast to other tissues examined, placenta and colon

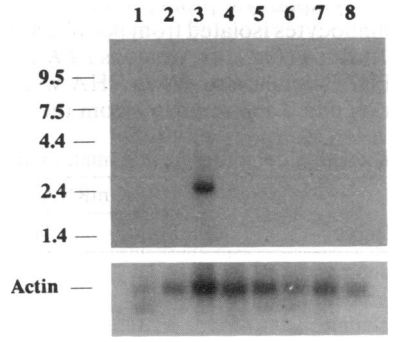


FIG. 2. Expression of *PLK* mRNA in adult human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas (lanes 1–8, respectively). Each lane contained 2 μg of poly(A)$^+$ RNA. Hybridization was done under high stringency with an antisense probe corresponding to aa 285–497 (*Upper*) and with an actin probe (*Lower*). Size markers at left are in kilobases.

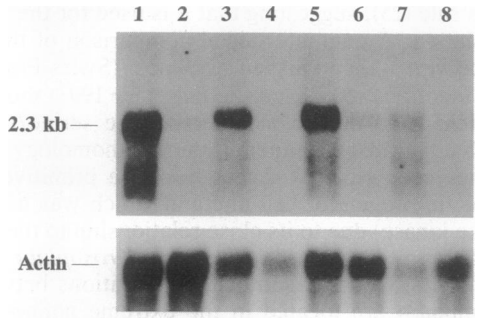


FIG. 3. Expression of *PLK* mRNA in human lung tumors. RNA was isolated from human adult normal and malignant lung tissues. Samples (20 μg) of total RNA were fractionated by electrophoresis through a 2.2 M formaldehyde/1% agarose gel. The blots were probed as in Fig. 2. Lanes 1, 3, 5, and 7, squamous-cell lung carcinoma; lanes 2, 4, 6, and 8, corresponding surrounding tissues from the same individual.

contain a high percentage of proliferating cells. This led to the assumption that expression of *PLK* might play a role preferentially or exclusively in mitogenic cells.

Human tumors of various origin were analyzed for *PLK* transcripts to test this hypothesis. High levels of PLK mRNA were found in most tumors of lung (Fig. 3), breast, esophagus, smooth muscle, stomach, colon, and non-Hodgkin lymphomas. However, a small percentage of lung and breast tumors exhibited little or no *PLK* mRNA expression. Table 1 summarizes the *PLK* mRNA analysis of 72 tumors. *PLK* mRNA was expressed in about 84% of 51 lung tumors screened. The corresponding surrounding lung tissues showed no or hardly detectable *PLK* mRNA expression which could be due to infiltrating neoplastic cells or inflammation. Southern blot hybridization of DNA from 10 tumors and corresponding tissues with a *PLK*-specific probe did not reveal any differences between normal surrounding and malignant tissues.

Further support for a role of *PLK* in mitogenic cells was provided by Northern blot analysis of human cell lines (Fig. 4). Strong expression of *PLK* mRNA was found in HeLa (epitheloid carcinoma of cervix), T-47D (ductal carcinoma of breast), endothelial, and lung epithelial cells. Slightly lower levels were observed in SK-BR-3 (adenocarcinoma of breast) and BT-20 (carcinoma of breast) cells. No cell line was found to be negative for *PLK* mRNA expression.

**Mitogen-Induced Expression of *PLK* mRNA.** PHA and interleukin 2 (IL-2) were used to induce proliferation of human $CD4^+$ lymphocytes. PHA activates the expression of IL-2 and its high-affinity receptor, which form an autocrine loop leading to lymphocyte proliferation.

Resting lymphocytes isolated from peripheral blood did not express *PLK* mRNA (Fig. 5*A*), whereas *PLK* expression was induced by PHA stimulation. When PHA was added to the lymphocytes on day 0 and removed from the culture on day

Table 1. *PLK* expression in malignant human tissues

| Tumor | PLK mRNA expression | |
|---|---|---|
| | Strong | Weak or none |
| Lung carcinoma | 43 | 8 |
| Leiomyosarcoma | 2 | 0 |
| Colon carcinoma | 3 | 1 |
| Non-Hodgkin lymphoma | 1 | 0 |
| Mammary carcinoma | 9 | 1 |
| Liver metastasis in lung | 1 | 0 |
| Stomach carcinoma | 2 | 0 |
| Esophagus carcinoma | 1 | 0 |
| Total | 62 | 10 |

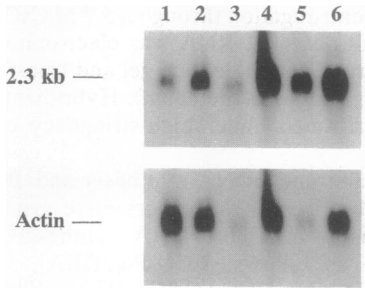


FIG. 4. Expression of *PLK* mRNA in human cell lines. Each lane contained 20 μg of total RNA from various proliferating human cell lines cultured in the presence of 10% fetal bovine serum. The RNA was hybridized with the same probes as in Fig. 2. Lane 1, BT-20; lane 2, T-47D; lane 3, SK-BR-3; lane 4, endothelial cell line; lane 5, lung epithelial cell line; lane 6, HeLa.

3, *PLK* mRNA was first observed between days 2 and 3, depending on the donor. It reached a maximum between days 3 and 4 and remained unchanged up to day 6 (Fig. 5*A*). The differing onset of *PLK* mRNA expression in lymphocytes was probably due to interindividual variations in the lengths of the initial lag phase and the $G_1$, S, and $G_2$ phases, which have been observed after mitogen stimulation (32).

Moreover, the influence of exogenous IL-2 on lymphocytes kept for 3 days in medium containing PHA was studied. While for T lymphocytes from some donors the PHA-induced autocrine IL-2 stimulation seemed to be sufficient for maximal *PLK* mRNA expression (Fig. 5*A*), T-lymphocyte cultures from other donors reached maximal *PLK* mRNA expression only after addition of IL-2 (Fig. 5*B*). We were unable to detect *PLK* mRNA in lymphocytes cultured for 3 or 4 days without PHA or IL-2 and in lymphocytes treated with IL-2 only (data not shown).

We also investigated the influence of serum on *PLK* mRNA expression in the epidermoid carcinoma cell line A-431. The cells were cultivated in RPMI 1640 medium with 10% fetal bovine serum before they were transferred to and kept in serum-free medium for various periods of time. Under these conditions *PLK* mRNA was still expressed, although the level of expression decreased with time (Fig. 6). After 7 days without serum, *PLK* mRNA expression was reduced by a factor of 5–10. When the cells were subsequently stimulated by the addition of 10% fetal bovine serum, *PLK* expression was enhanced within 1 day.

***PLK* mRNA Expression Is Prevented by Cycloheximide.** Cycloheximide, an inhibitor of protein synthesis, was used to study the influence of *de novo* protein synthesis on the level of *PLK* transcripts to give further insight into the role of PLK during cell division. Superinduction of transcription under

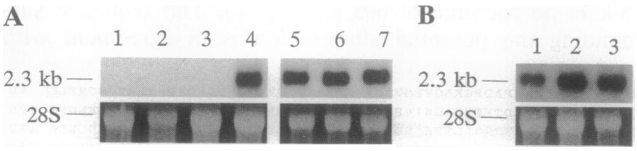


FIG. 5. Mitogen induction of *PLK* mRNA expression in human lymphocytes. Human peripheral lymphocytes were isolated and cultivated as described (18). Each lane contained 7 μg of total RNA. Ethidium bromide-stained gels (with position of 28S rRNA) are shown below the autoradiogram to indicate the amount of RNA loaded. (*A*) *PLK* mRNA expression in resting lymphocytes (lane 1), in lymphocytes stimulated with PHA for 1 day (lane 2), 2 days (lane 3), and 3 days (lane 4), and in lymphocytes stimulated with PHA for 3 days and then cultured without PHA for 1 (lane 5), 2 (lane 6), or 3 (lane 7) additional days. (*B*) Influence of IL-2 on the expression of *PLK* mRNA. T lymphocytes were stimulated for 3 days with PHA as above. After removal of PHA the cells were cultured with IL-2 for 1 day (lane 1), 2 days (lane 2), or 3 days (lane 3).

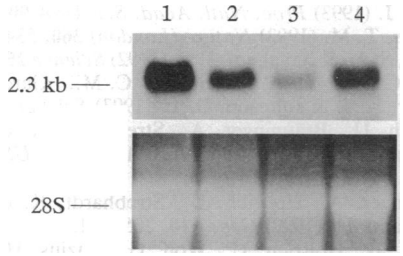


FIG. 6. Influence of fetal bovine serum on *PLK* mRNA expression in A-431 cells. A-431 cells were kept under serum starvation for 5 or 7 days. After 7 days of starvation fetal bovine serum was added (10%). RNA was prepared from adherent cells. Each lane contained 20 μg of total RNA. Lane 1, A-431 cells in RPMI 1640 with 10% fetal bovine serum; lane 2, A-431 cells after serum starvation for 5 days; lane 3, A-431 cells after serum starvation for 7 days; lane 4, A-431 cells after serum starvation for 7 days and subsequent stimulation with serum for 1 day.

the influence of cycloheximide has been described for a number of genes involved in early growth response (29). T lymphocytes were stimulated for 1 day with PHA and then treated for 24 hr with cycloheximide. Induction of *PLK* mRNA in T lymphocytes occurred between days 2 and 3 and was prevented by cycloheximide (Fig. 7).

**Down-Regulation of *PLK* mRNA Expression in Human Macrophages.** Peripheral blood-derived macrophages are nonproliferating cells when kept in culture for 15 days. Depending on the donor, two types of macrophage cultures were observed: those which did not express *PLK* mRNA, consistent with the hypothesis that *PLK* is predominantly expressed in mitogenic cells, and those with a low level of *PLK* mRNA (three out of six cultures). Macrophage cultures positive for *PLK* transcripts were used to determine the influence of bacterial lipopolysaccharide on *PLK* mRNA expression. The macrophages were cultured for 15 days and then treated with lipopolysaccharide for various times. *PLK* mRNA expression was shut down within 24 hr of lipopolysaccharide treatment (Fig. 8).

## DISCUSSION

In this study we identified a member of the family of serine/threonine kinases which appears to be the human counterpart of mouse Plk.

Several lines of evidence suggest that *PLK* mRNA expression is associated with cellular proliferation. (*i*) The expression was most prominent in normal proliferating human adult tissues (placenta and colon). (*ii*) Compared with surrounding normal tissues, various types of tumors (lung, breast, colon, stomach, smooth muscle, and esophagus) very often showed high levels of *PLK* mRNA. Small-cell lung carcinomas grow especially fast (33). Interestingly, the two small-cell lung

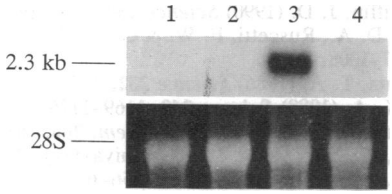


FIG. 7. Influence of cycloheximide on *PLK* mRNA expression in mitogen-activated lymphocytes. Lymphocytes were stimulated for 1 day with PHA. Half of the culture was supplemented with cycloheximide at 75 μM. After 24 hr, RNA was isolated. Lane 1, resting lymphocytes; lane 2, lymphocytes stimulated 1 day with PHA; lane 3, lymphocytes stimulated 2 days with PHA; lane 4, lymphocytes stimulated for 1 day with PHA and cultured 1 additional day with cycloheximide and PHA.

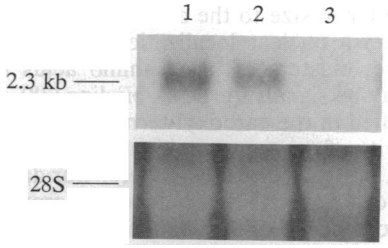


FIG. 8. Repression of *PLK* mRNA expression in human macrophages by lipopolysaccharide. Macrophages were cultured for 15 days (lane 1) and then treated for 6 hr (lane 2) or 24 hr (lane 3) with lipopolysaccharide (100 ng/ml, *Salmonella abortus-equi*, courtesy of C. Galanos, Max Planck Institute, Freiburg, Germany). Each lane contained 20 μg of total RNA.

carcinoma samples examined showed much higher *PLK* mRNA expression than non-small-cell lung carcinomas (data not shown). (*iii*) PLK mRNA was found in all immortalized cell lines examined. *PLK* mRNA expression in A-437 cells was stimulated by serum. (*iv*) In cultured lymphocytes *PLK* mRNA expression was induced by mitogen (PHA/IL-2) treatment. (*v*) Nonproliferating macrophages exhibited little or no *PLK* mRNA.

However, some of the nonproliferating terminally differentiated macrophage cultures showed *PLK* mRNA expression and ≈15% of the tumor samples which were assumed to be proliferatively active tissues did not. Regarding cultured macrophages, motion pictures have revealed endogenous mitosis in rare cells (34). This observation could indicate that *PLK* mRNA expression correlates with mitotic events, but not necessarily with cell division. Still, a specific functional role of PLK in macrophages cannot be ruled out. Treatment of macrophages with lipopolysaccharide, which stimulates macrophages to produce large amounts of various cytokines (35), shut down *PLK* mRNA expression completely within 1 day.

We assume that those tumor samples which were negative for *PLK* mRNA were derived from a portion of the tumor with a low level of proliferation or from a resting neoplasm (cancer dormance). Future investigations will have to elucidate the nature of the phenomenon by detailed methods. If this holds true in further studies, *PLK* expression may be an excellent marker for the degree of malignancy of the tumor.

Analysis of PLK-related genes in other species also suggests their involvement in cellular proliferation. The polo gene from *D. melanogaster* is expressed in tissues with high proliferative activity. In larvae homozygous for a polo mutation, certain cells are blocked in mitosis and are unable to proliferate to become adult structures. The murine counterpart *Plk* was found to be expressed in fetal liver, kidney, and brain, but no *Plk* mRNA was detected in corresponding adult tissues (26). According to these data the function of Plk might be important for proliferation, but a functional or morphogenetic role for mouse embryogenesis cannot be ruled out. Our data provide evidence for a correlation of *PLK* mRNA expression and the proliferative activity of cells.

Serine/threonine kinases which participate in cellular proliferation are localized in the cytoplasm or the nucleus. Interestingly, the analysis of the PLK amino acid sequence revealed several putative NLS sequences. Such sequences facilitate in some way the selective entry of certain proteins into the nucleus through the nuclear pore complex (30, 31). In the case of simian virus 40 large tumor antigen a NLS sequence of five basic amino acids (KKKRK) is sufficient to direct the protein to the nucleus (36). In the case of the human papillomavirus type 16 L1 protein, the NLS consists of two short clusters of basic amino acids (37). The clusters identified in the PLK protein consist of basic amino acids as well

and are similar in size to the above-mentioned motifs. This might predict a nuclear localization for the PLK as well. Furthermore, clusters of basic amino acids and aromatic residues may be signals for DNA binding (38). Those motifs were identified in the carboxyl-terminal portion of PLK.

The activity of most protein kinases is regulated by phosphorylation or by second messengers. Our data suggest that expression of *PLK* mRNA is induced by growth-stimulating agents. Two other kinases, cdc2 kinase and Snk, are regulated on the transcriptional level as well (29, 39).

A close link of *PLK* mRNA expression to mitotic activity was detected in lymphocytes. T cells stimulated by foreign antigens such as PHA enter a program of cellular activation leading to *de novo* synthesis of IL-2 and its high-affinity receptors (40–43). Interaction of IL-2 with its induced receptor triggers cellular proliferation culminating in the emergence of the effector T cells that are required for full expression of the immune response. Whereas no *PLK* mRNA was found in resting T cells, PHA/IL-2 stimulation was accompanied by increases in *PLK* mRNA. It was recently proposed that IL-2 is linked to two alternative intracellular signaling pathways: one involving an unknown mechanism leading to c-*myc* activation and entrance of the cell into S phase and one involving cytoplasmic protein-tyrosine kinase activation followed by induction of c-*jun* and c-*fos* (44, 45). It will be interesting to see in which of these pathways PLK plays its role.

Mitogen-stimulated lymphocytes cultivated in the presence of cycloheximide did not show PLK mRNA, in contrast to the superinduction effect of cycloheximide on several early growth-response genes. The lack of *PLK* mRNA in those cells might have been due to reduced transcription, suggesting that *PLK* is not an early growth-response gene, but could also have resulted from decreased stability of the *PLK* mRNA.

In summary, we found *PLK* mRNA expression to be associated with proliferative activity. We have provided evidence for the mitogen-inducible transcription of *PLK*. Furthermore the presence of various potential NLS sequences suggests that PLK is located in the nucleus. It might even interact with nucleic acids directly, combining kinase activity and DNA-binding properties in one molecule.

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