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Human CDK10 Gene Isoforms

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The CDK10/PISSLRE gene has been shown to encode two different CDK-like putative kinases. The function(s) of the gene products are unknown, although a role at the G2/M transition has been suggested. We characterised two novel cDNAs. CDK10 mRNA quantity was not found to be correlated with cell proliferation status in HeLa or WI38 cell cultures or in human tissues. Relative levels of the four CDK10 isoforms were studied by RT-PCR, of which three were principally expressed. The two initially cloned isoforms predominated in human tissues, except in brain and muscle. Relative isoform levels did not vary during the cell cycle in culture, except when cells entered into the cell cycle. Finally, the predominant isoforms were shown to have different translation initiation sites and to have different subcellular distribution, due to an alternatively spliced nuclear localisation signal.

Key Words: **PISSLRE; alternative splicing; isoform; NLS.**

Cyclin-dependant kinases (CDKs) were first described as key regulators of the eukaryotic cell division cycle (1–3). CDK1 controls G2/M transition and CDK2, CDK3, CDK4, and CDK6 are implicated at G1/S. CDKs are also implicated in processes other than control of the cell cycle. For instance, CDK5 is required during neural differentiation, CDK7, the kinase subunit of TFIIH, is implicated in cell cycle control and regulation of transcription, and CDK8 and CDK9 are linked to transcription control (4–7). New members of the CDK family have been isolated according to homologies in specific amino acid sequences and usually named according to their sequences in the conserved "PSTAIRE" domain (e.g., PCTAIRE, PISSLRE, and PITSLRE) (8). Most of these new CDKs do not have a clearly established role or cyclin partner, and could enlarge the cell functions of this kinase family (8).

PISSLRE was identified using two independent PCR-based strategies (9, 10). Two cDNAs were described that are identical in their sequence encoding the putative kinase core but divergent in their 5′- and 3′-extremities. This suggested the possibility of differential expression of the PISSLRE gene, regulated by alternative splicing. Indeed, sequencing of the gene has recently confirmed that the two isolated cDNAs are derived from a single gene (11). This gene has been renamed *CDK10,* although no cyclin associated with the putative kinases has yet been reported.

A study by Li *et al.* using a dominant negative mutant and an antisense vector led to the suggestion that CDK10 was implicated at the G2/M transition of the cell cycle (12). However an exclusive role of CDK10 in the cell cycle seems unlikely, since the mRNA is expressed even in non-proliferative tissues (9, 10). The functions of CDK10 therefore remain to be fully characterised.

This study focuses on CDK10 isoforms. Additional alternatively spliced forms in the 5′ region of the messenger were found. The relative mRNA levels of the CDK10 isoforms were shown to be tissue-dependent and to vary in tissue culture cells as a function of cell proliferation status rather than cell cycle stage. Finally, the subcellular distribution of proteins corresponding to the two most abundant CDK10 mRNAs was found to be different.

MATERIALS AND METHODS

Cell culture and transfection. Primary human fibroblast WI38 cells and HeLa cells were cultured at 37°C (5% CO_2) in Dulbecco

The HGMW-approved symbol for the PISSLRE gene is cyclin-dependent kinase 10 (CDK10). Sequence data from this article have been deposited in the GenBank Data Library under Accession No. AF153430.

Abbreviations used: CDK, cyclin-dependent kinase; GFP, green fluorescent protein; ORF, open reading-frame; NLS, nuclear localisation signal; RT, reverse transcription; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; MW, molecular weight.

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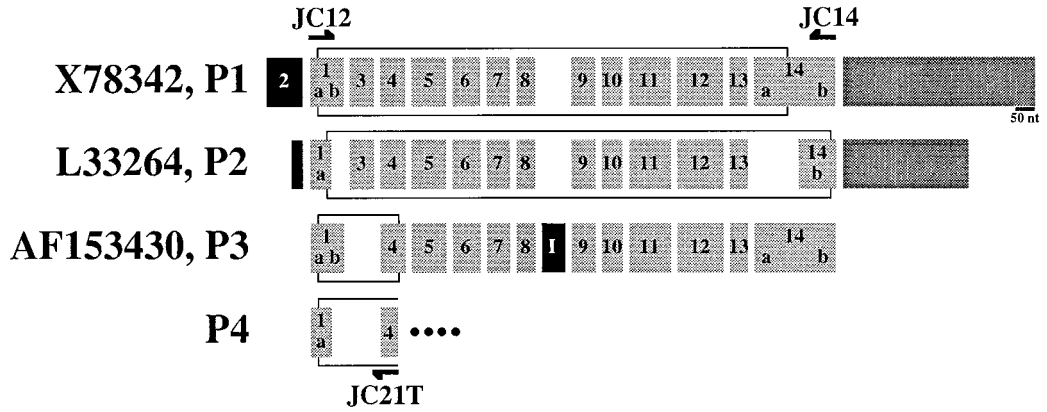

FIG. 1. Structure of four transcripts of the CDK10 gene. The bar beneath the 3′-end of the X78342 transcript corresponds to 50 nucleotides. Light-grey boxes (exons) are numbered according to the nomenclature suggested by Crawford *et al.* (11) and alternatively spliced according to the isoform (P1–P4). Dark-grey boxes in X78342 and L33264 transcripts are 3′-end untranslated sequences. Black boxes are intron 8 (P3 transcript) and specific 5′-extremities of the X78342 and L33264 transcripts (X78342 contains exon 2). JC12, JC14, and JC21T primers used in the various cloning procedures are indicated (not to scale). The P4 structure downstream from the JC21T primer is unknown, as indicated. Open boxes symbolise the open reading-frame corresponding to each isoform.

modified Eagle's medium with 10% foetal calf serum. Transfections were carried out using Exgen500 (Euromedex), according to the manufacturer's recommendations.

cDNA cloning. Polymerase chain reaction (PCR) was carried out with the Advantage-GC cDNA PCR kit (Clontech), using primers JC12 (5′-CCAGCGCTCGGCATGGCGGAGCCAGA-3′) and JC14 (5′-GGGAGGAAGTCAGTGGAGGATCTTCCCAGCC-3′), and the human lung MATCHMAKER cDNA library (Clontech) as the template. PCR products of 1–1.2 kb were cloned with the pMOSBlue T-vector kit (Amersham) and sequenced. 5′-RACE was carried out using version 2.0 5′ of the RACE System (Gibco BRL) according to manufacturer's instructions and oligonucleotides JC10 (5′-GTCGATGC TGGTGGTCTGCGTGGTGGT-3′) (WI38 fibroblast total RNA reverse transcription), JC36 (5′-GCCAGGCCGAAATCCGCT-3′) (first PCR), and JC21T (5′-CTTCAGTGCGACAATCTCAT-3′) (second nested PCR).

Plasmids. P1 and P2 ORFs were inserted into a Quiagen plasmid pQE32 and sequenced. These ORFs were subsequently excised and inserted into a phGFP plasmid to yield phGFP-P1 and phGFP-P2 (phGFP is based on Invitrogen pcDNA3). All other plasmids (all based on pcDNA3) were constructed using PCR or PCR-mutagenesis, and sequenced.

Translation assays. HeLa cells (10^5) were collected 24 h after transfection, resuspended in 20 μl SDS sample buffer, and boiled for 10 min. GFP fusion proteins were detected by Western blotting using monoclonal antibody against GFP (Clontech) after 8% SDS–PAGE. *In vitro*-translated ^{35}S-labelled proteins (TNT T7 Quick Coupled Transcription/Translation System, Promega) were immobilized on Ni-NTA Superflow resin (QUIAGEN) in Ni20 buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 20 mM imidazole), washed 3 times in Ni50 buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 50 mM imidazole), resuspended in 20 μl SDS sample buffer, boiled for 10 min, subjected to 8% SDS–PAGE, and finally revealed by autoradiography.

RNA isolation and RT-PCR analysis. Total mRNA from 10^6 to 10^7 cells was extracted using the RNA NOW reagent (Ozyme) and treated with DNase. RNA quality was checked by electrophoresis in 1.5% agarose/formaldehyde denaturing gel. cDNA was prepared from 5 or 10 μg total RNA, using oligo(dT)$_{12-18}$ primer and Moloney murine leukaemia virus reverse transcriptase (Gibco BRL). cDNA amplification by PCR was carried out using CDK10-specific primers. For radioactive RT-PCR reactions, primer JC21T was labelled with [^{32}P]-γ-ATP prior to PCR. PCR products were resolved on a 6% non-denaturing polyacrylamide gel and either autoradiographied (Kodak Biomax film and amplifying screens) or analysed with a β-Imager 1200 (Biospace Instruments). For tissue-pattern expression, PCR was carried out on 24 cDNAs normalised against β-actin and serially diluted over a 4-log range, using the human RAPID-SCAN gene expression panel (Origene Technologies), as described by the manufacturer.

Quantification of CDK10 mRNA. Northern blot analyses were carried out with a P2 cDNA probe that hybridised with all CDK10 isoforms. Expression in human tissues were accomplished using the RNA Master Blot (Clontech), which is normalised for the amount of spotted purified polyadenylated mRNA.

Cell cycle synchronisation and analysis. WI38 fibroblasts were serum-starved for 48 h, serum was added (t_0), and cells were collected for analysis at various times. HeLa cells were synchronised with a double thymidine block (2 mM thymidine in DMEM/FCS for 17 h, followed by growth in drug-free medium for 9 h, and then 2 mM thymidine for 15 h). After washing in drug-free medium (t_0), cells were harvested at different times for further analysis. Blocks in specific phases of the cell cycle were obtained using either aphidicolin 5 μg/mL or nocodazole 250 ng/mL for 20 h. Gamma-irradiation, using a ^{137}Cs source, and cell cycle analysis were carried out as previously described (13).

Microscopy. Cells were grown and transfected on glass coverslips briefly washed in PBS, incubated upside-down on a drop of H33342 (5 μg/ml in PBS) for 5 to 30 min, and mounted on a glass slide. GFP and H33342 fluorescence were observed with a Leica DM-RB microscope, respectively using FITC and DAPI filters with an oil-immersion 63× objective. Images were taken on Kodak EliteChrome 400 film with the Leica DM-RD platform, then scanned.

RESULTS

Characterisation of Alternative Splicing in the 5′ Region of the CDK10 mRNAs

During cloning of the two previously characterised cDNAs (GenBank X78342 and L33264, respectively named isoforms P1 and P2 in this study), new CDK10 cDNAs were isolated (Fig. 1). A novel isoform was first detected using a PCR screen on a cDNA library of WI38

human primary fibroblasts and primers based on the most-5′-and 3′-common regions of X78342 and L33264 (primers JC12 and JC14). This novel 1237 base-pair cDNA (GenBank AF153430), designated isoform P3 in this study, was identified as 8 out of 20 clones isolated from two independent screens. The 5′-primer (JC12) contained the putative P1 initiating codon. We checked whether the 5′-ends described in X78342 and L33264 (9, 10) could be found upstream from the P3 sequence. Polymerase chain reactions based on specific 5′-untranslated sequences of P1 or P2 and a 3′-P3-specific primer were carried out, but never yielded a specific product (data not shown). To identify putative upstream nucleotides, a 5′-RACE was carried out using mRNA from WI38 cells. None of the P3 clones extended any further upstream from exon 1a. The novel P3 isoform is therefore composed of exons 1a, 1b, 4–8, intron 8, and exons 9–14b, according to the nomenclature suggested by Crawford *et al.* (Fig. 1). The 5′-RACE also revealed another cDNA called P4 in this study (Fig. 1). The 5′-end of P4 links exon 1a and exon 4. Since P4 was barely detectable in subsequent RT-PCR experiments, we did not attempt to clone full-length P4 cDNA (see below and discussion).

Alternative Splicing in the 5′-Region of CDK10 mRNA Modifies the Translation Initiation Site

Because of alternative splicing in the 5′-region of CDK10 mRNA which creates a frameshift, it has been suggested (but not demonstrated) that translation initiation is different for the P1 and P2 isoforms, yielding different full-length putative kinases. For P1-cDNA, the first methionine present in exon 1a is encompassed in a perfect Kozak consensus and has been proposed by Draetta *et al.* (9) to be the initiating codon (light grey box, Fig. 2A). To confirm this assumption, we used constructs in which a GFP tag was added in frame with the putative P1 ORF, either at the N- or C-terminus (respectively yielding GFP-P1 and P1-GFP). These constructs allowed both *in vitro* translation and *in vivo* expression of the fusion proteins. ^{35}S-labeled, *in vitro*-translated GFP-P1 and P1-GFP proteins and crude extracts of human cells transiently transfected respectively with GFP-P1 and P1-GFP constructs were subjected to SDS–PAGE and respectively revealed by autoradiography and Western blotting with anti-GFP antibodies. As shown on Fig. 2B, *in vivo* and *in vitro* translation products co-migrated at a size compatible only with the initiation of translation at that first methionine.

For P2-cDNA, Grana *et al.* (10) have used *in vitro* translation to show that a putative kinase could be translated starting at a valine encoded in exon 1a (dark grey box, Fig. 2A). Following the same strategy used in the P1 study, we constructed plasmids in order to express P2-GFP and GFP-P2. As shown in Fig. 2B, *in vitro*-translated and *in vivo*-transiently expressed P2-GFP and GFP-P2 co-migrated at a size compatible with the initiation of translation at the expected valine.

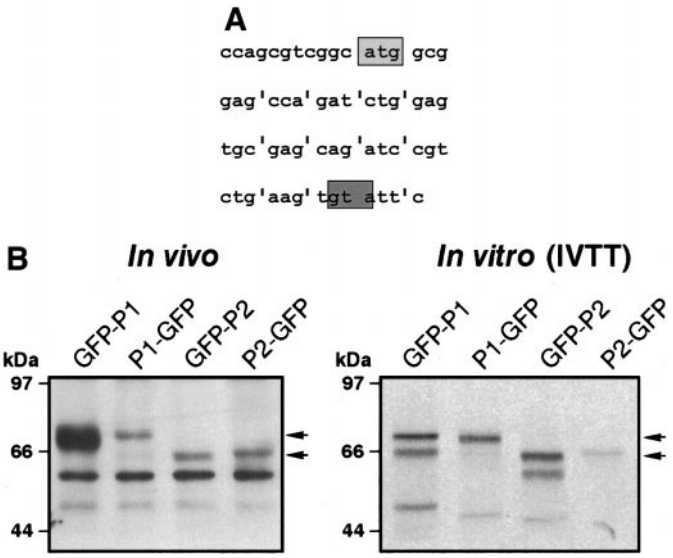

FIG. 2. Alternative splicing in the 5′-region of CDK10 mRNA modifies the putative translation products. (A) Nucleotide sequence of exon 1a. The light-grey box indicates the first ATG, located in a perfect Kozak consensus sequence and is the initiating codon in P1. The dark-grey box indicates the GTA codon encoding the valine that allows initiation of translation in P2. (B) *In vitro* and *in vivo* initiation of translation. Left panel: whole extracts of HeLa cells transfected with GFP-P1/P2 and P1/P2-GFP constructs separated by 8% SDS–PAGE and subjected to Western blotting with a monoclonal anti-GFP antibody. Right panel: ^{35}S-methionine labelled *in vitro* coupled transcription and translation products of plasmids P1/P2-GFP and GFP-P1/P2 separated by 8% SDS–PAGE and revealed by autoradiography. MW: P1-GFP = 68.7 kDa, GFP-P1 = 69.3 kDa, P2-GFP = 63.4 kDa, GFP-P2 = 64 kDa. Arrows indicate bands corresponding to P1 and P2 products.

We have thus shown that translation can be effectively initiated at a valine in the case of P2 and at an upstream methionine in the case of P1. Since alternative splicing between exons 13 and 14b also changes the reading frame at the end of the ORF, CDK10 P1 and P2 mRNAs yield putative kinases with strikingly different N- and C-termini.

Relative Levels of Alternatively Spliced Isoforms Are Regulated

An RT-PCR experiment was designed and carried out in order to study the relative levels of the isoforms resulting from splicing between exon 1a and exon 4. We first compared exponentially growing and confluent HeLa and WI38 cells (Fig. 3A). Northern blot analysis did not show significant variation in CDK10 total mRNA between growth-arrested and exponentially growing WI38 cells (data not shown). As determined by RT-PCR, in HeLa cells, P2-like ends predominated both at confluence and in exponentially growing cells,

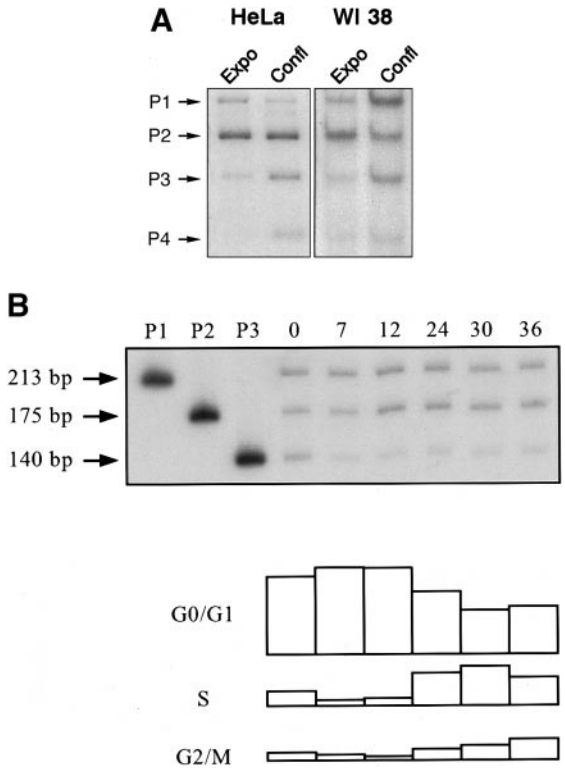

FIG. 3. Relative levels of alternatively spliced isoforms are regulated. RT-PCR assay amplifying the region between exons 1a and 4 of CDK10 (primers JC12 and JC21T, Fig. 1). P1-like ends = 213 bp, P2-like ends = 175 bp, P3-like ends = 140 bp, P4-like ends = 102 bp. (A) CDK10 isoforms and the proliferative status of tissue-culture cells. Autoradiography of PCR products from confluent (Confl) and exponentially growing (Expo) HeLa cells and WI38 human primary fibroblasts. Electrophoresis on a 6% non-denaturing polyacrylamide gel. (B) CDK10 isoforms in synchronised WI38 fibroblasts. RT-PCRs of mRNA extracted from WI38 fibroblasts synchronised by serum starvation at various times after serum addition (0, 7, 12, 24, 30, and 36 h). DNA content of harvested cells was determined by FACS analysis and percentage of cells with G0/G1, S, and G2/M DNA content are shown as histograms. Representative results of 2 independent experiments. (C) CDK10 isoforms in various human tissues. RT-PCR of "human RAPID-SCAN gene expression panel" (Origene Technologies). PCR products were resolved on 2.5% metaphore agarose and dyed with ethidium bromide. Representative results of 4 experiments.

whereas the situation was balanced between P1- and P2-like ends in primary fibroblasts. In both HeLa cells and primary fibroblasts, the relative level of P3 like-ends was higher in confluent than in exponentially growing cells (30%, versus 10–15% of all CDK10 5′-ends). P4-like ends were barely detectable, consistently amounting to less than 5% of the total CDK10 mRNA (Fig. 3A and data not shown).

These results and the fact that CDK10 had been proposed as being involved in the G2/M transition of the cell cycle (12) prompted us to examine the relative levels of CDK10 mRNA during the cell cycle. WI38 cells were synchronised by serum starvation followed by serum re-addition. As the cells resumed cycling, no significant change was detected except for a sharp relative decrease in P3-like ends to the profit of P1- and P2-like ends within the first 7 h after serum stimulation (Fig. 3B). HeLa cells were also synchronised with a double thymidine block. No change in the mRNA profile was detected as the cells resumed progression through the cell cycle (data not shown). Exponentially growing HeLa and WI38 cells were then blocked in early S-phase by aphidicolin, in G2 phase by 3 or 10 Gy of gamma-irradiation, and in M phase by nocodazole. No change in the 5′-end CDK10 profiles was detected (data not shown). Progress in the cell cycle therefore did not affect the relative levels of CDK10 isoforms, except when the cells left quiescence.

Levels of CDK10 total mRNA and relative levels of CDK10 mRNA 5′-ends were then determined in differentiated human tissues. Relative levels were determined by semi-quantitative RT-PCR in 24 human tissues. Almost identical patterns of expression were observed in most tissues, with a predominance of the P1 isoform (Fig. 3C), such as in WI38 at confluence (Fig. 3A). However, brain, muscle, and to a lesser extent, heart, displayed high relative levels of mRNAs with P3-like ends (Fig. 3C). The total level of CDK10 was determined by Dot blot analysis in 48 tissues (data not shown). CDK10 mRNA was found to be ubiquitously expressed, although up to 8-fold variations were noted. The highest levels were found in pancreas, pituitary gland, lung, prostate, liver, placenta, ovary, and testis. The lowest levels were found in colon, PBL, bone marrow, heart, brain, muscle, and spleen. Intermediate levels were found in foetal tissues (brain and liver), as well as in kidney, small intestine, and uterus.

P1 and P2 Putative Kinases Are Targeted to Different Cellular Compartments

Since P1 and P2 were the most abundant CDK10 messengers, we determined the cellular localisation of these putative kinase isoforms. In the absence of antibodies specifically recognising CDK10 proteins, localisation of GFP-labelled proteins was studied. In HeLa cells, GFP-P1 and P1-GFP proteins were strictly nuclear, whereas the GFP control, GFP-P2, and P2-GFP proteins were evenly distributed throughout the cell (Fig. 4A). A slight nuclear exclusion of GFP-P2 and P2-GFP proteins was observed in a small percentage of HeLa cells (Fig. 4A; the size of the fusion protein, 64 kDa, is at the upper limit for passive diffusion through the nuclear pore). Similar results were obtained with the human primary fibroblast WI38 and MCF7 breast adenocarcinoma cell lines.

Since P1 and P2 differ only in their extremities, these regions were likely to be responsible for the specific nuclear localisation of P1. The P1 C-terminal sequence, encoded by exon 14a, contains two stretches of basic amino-acids separated by 10 residues that might

correspond to a bipartite nuclear localisation signal (NLS) (14). Fusion of the open reading-frame (ORF) of exon 14a to the GFP C-terminus was sufficient to target GFP to the nucleus (Fig. 4C). Exon 14a therefore contains an autonomous NLS. The basic residues were mutated to alanine, yielding the KR344AA and KR356AA mutants. One or the other of these mutations abolished the specific nuclear localisation of GFP-P1 (Fig. 4C), demonstrating that these residues do indeed constitute a classical bipartite nuclear localisation signal.

DISCUSSION

A number of genes that play an important role in cell fate are subjected to regulation by alternative splicing of their pre-mRNA. These include several CDK regulator cyclins (E, D1, and B) (15–17), phosphatase CDC25B (18), and p16 INK4a/p19^{ARF} (19), as well as E2F (20), members of the p53 family (21, 22), and Bcl-x (23). Isoforms can behave in dramatically different ways and have different functions. For example, Bcl-x_L protects cells from apoptosis, whereas Bcl-x_S is pro-apoptotic (23).

Since the CDK10 gene is expressed as several isoforms, it was important to identify them and determine which of them were the most expressed. P1 and P2, corresponding to putative kinases, were the first described (9, 10). Crawford *et al.* recently described the genomic structure of CDK10, showing that P1 and P2 derive from the same gene by alternative splicing (11). Using 5′-RACE, they also obtained alternatively spliced 5′-end sequences corresponding to P1, P3, and P4 (but not P2). In addition to that group's data, we describe here a 3′-sequence of P3 cDNA. Although both Crawford *et al.* and our group found the 5′-end of the P4 isoform in 50% of the 5′-RACE experiments, it was barely detectable in an RT-PCR assay based on poly-dT reverse-transcribed cDNAs (Fig. 3A). P4 could thus be a non-polyadenylated transcript. The RT-PCR assay that we used to study relative isoform levels detects splicing only between exons 1a and 4. We can not exclude that regions between exons 4 and 13 are subject to alternative splicing, which, in combination with splicing between exons 1a and 4 and between exons 13 and 14b, would yield a number of other isoforms. However we did not find any evidence of additional alternative splicing in the 20 clones identified at the outset of our study. We therefore think it likely that the P1, P2, and P3 cDNAs described in Fig. 1 are the most relevant and abundant CDK10 gene transcripts.

The absolute levels of CDK10 mRNA did not correlate with the proliferative status of tissues or cells. Indeed, we found total quantities of CDK10 mRNA to be similar in quiescent and exponentially growing tissue culture cells. Foetal tissues and bone marrow, which have the highest mitotic index, also showed average or low levels of CDK10 mRNA. Our results complement those of Grana *et al.* and of Brambilla *et al.*, who have shown CDK10 mRNA to be ubiquitously expressed (9, 10). The three mRNAs corresponding to P1, P2, and P3 were present in every tissue and cell line we examined (Fig. 3). There was a large increase in the relative level of the P3 isoform in growth-arrested culture cells and in some tissues, such as brain and muscle. *In vitro* exponentially growing cells showed a predominance of P2. In normal tissues, P1 and P2 were the more abundant isoforms, except for brain and muscle. It is therefore likely that each CDK10 isoform carries out specific functions.

Alternative splicing of CDK10 gene transcripts results in dramatically different translation products. Indeed, our results are consistent with P1 initiating codon as a methionine in exon 1a, and P2 initiating codon as a downstream valine (Fig. 2). Every cDNA isolated from CDK10 displays exon 1a at its 5′-end. The ribosome-scanning translation model thus predicts that transcription should start at the same methionine codon in every isoform that it does in P1 cDNA. The absence of exon 1b in P2 would result in a short, 21-amino acid polypeptide, and it has been shown that translation of a "mini-cistron" could be followed by translation initiation at a codon downstream from the first methionine codon (24). Alternatively, initiation of translation at the valine codon in P2 may be due to "leaky scanning," particularly for P2 mRNAs with short 5′ extensions. The P1 and P2 translation products are putative protein kinases (P1 = 360 amino acids, predicted MW = 41 kDa; P2 = 316 amino acids, predicted MW = 358 kDa). The P1 and P2 proteins would share a common kinase core (corresponding to the highly conserved CDK core domain) and would differ in their amino- and carboxy-termini. For the P3 isoform, the translation product starting at the same methionine codon as in P1 would be a 42-amino acid polypeptide. Downstream initiation at the P2 valine codon would yield a 23-amino acid polypeptide, and initiation at the next downstream methionine codon would yield a 5-amino acid peptide. We therefore suggest that the P3 cDNA is likely to be translated into the 42-amino acid protein. This small protein (predicted MW: 4.905 kDa), the first 29 amino acids of which would be strictly identical to the N-terminus of the P1 kinase, would not contain the kinase core domain and should therefore be devoid of any kinase activity. These hypothetical P3 polypeptides do not belong to any protein family. We are currently seeking proof of the actual existence of the P3 polypeptide prior to further study of this isoform.

The P1 protein was shown to have a nuclear localisation signal (NLS) in its carboxy terminus (Fig. 4). CDK10 cDNAs have been cloned in various species. The NLS sequence is partially conserved in distantly related species such as *Haematobia irritans*

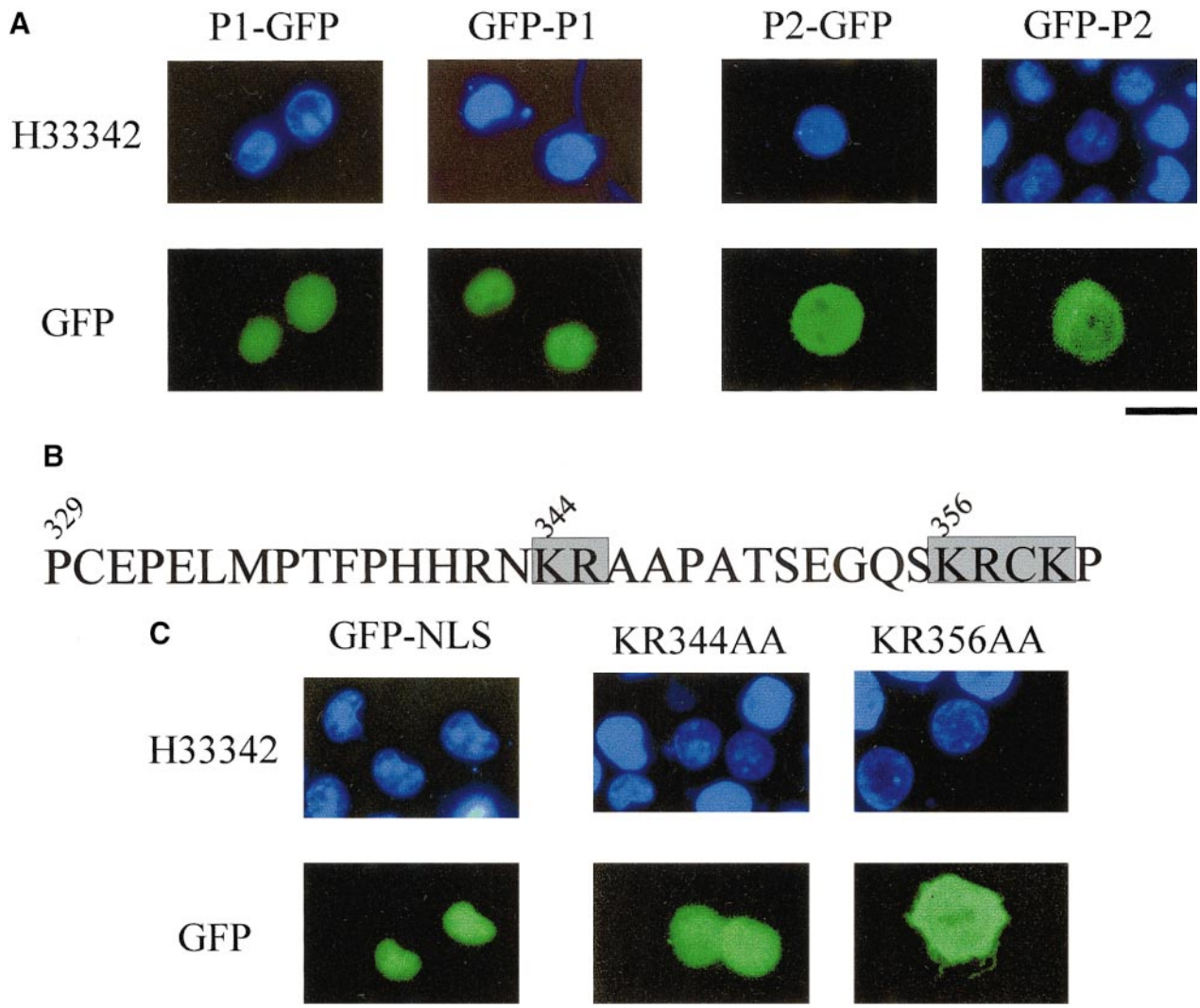

FIG. 4. The P1 and P2 CDK10 isoforms are targeted to different subcellular compartments. HeLa cells transfected with GFP-labelled CDK10-P1/P2 constructs. Localisation was assessed 24 h after transfection. Nuclei were labelled with the H33342 DNA staining reagent. (A) P1-GFP and GFP-P1 are nuclear proteins. Cells transfected with wild-type P1 and P2 isoforms in N- and C-terminal fusion with GFP. The bar beneath GFP-P2 corresponds to 10 μm. (B) Nuclear localisation signal. Amino acid sequence encoded by exon 14a. Highlighted basic amino acids constitute the bipartite NLS. (C) NLS mutagenesis. Cells transfected with GFP-NLS (left panel) and with GFP-P1 mutant (two right panels) constructs. The GFP-NLS construct corresponds to additions of the whole 14a exon to the C-terminus of GFP.

(GenBank AF034643), *Drosophila melanogaster* (GenBank BAA03886), and *Bombyx morii* (GenBank BAA21484), whereas it is identical in murine cDNA (GenBank AI640100). Alternative splicing as a mode of regulation for the presence of an NLS has already been shown for other proteins, such as the E2F transcription factor and $p58^{GTA}$, the closest CDK10 homologue (20, 25). The differential cellular localisation of P1 and P2 provided by the presence of an NLS in P1 again suggests different roles for these two isoforms.

While the functions of the CDK10 isoforms remain unknown, they should be important for cell fate, since the gene is expressed in every tissue tested so far (this study and (9, 10)). P2 has been described by Li *et al.* as necessary during the G2/M phase (12). Indeed, overexpression of a dominant negative mutant or an antisense construct led to cell accumulation in G2/M (12). However, we showed here that there is no correlation between CDK10 mRNA levels, the cell cycle, and cell proliferation. The accumulation of cells at G2/M could thus be indirect, for instance, by activation of a checkpoint, as reported for the closest CDK10 homologue, $p58^{GTA}$ (26).

In conclusion, three main isoforms of CDK10 mRNA were detected in normal cells. P1 and P2 mRNAs encode putative kinases and are present in all cells and tissues. P3 mRNA might encode a short polypeptide and is found to be the most transcribed isoform in growth-arrested cell cultures and in such tissues as brain and muscles. Subcellular distribution of CDK10

gene products depends on the alternative splicing of messengers. The study of CDK10 functions will therefore have to take into account all isoforms of the gene.

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