Identification and characterization of a 14 kDa human protein as a novel parvulin-like peptidyl prolyl *cis/trans* isomerase

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Abstract A second member of the parvulin family of peptidyl-prolyl *cis/trans* isomerases was identified in a human lung cDNA library. The gene encoded a protein named hPar14 that has 131 amino acid residues and a molecular mass of 13 676 Da. Sequence comparison showed 34.5% identity to *E. coli* Par10 and 34% identity to human Pin1 (*h*Par18) within a C-terminal region of 87 or 120 amino acid residues, respectively. In comparison to the *E. coli* Par10, *h*Par14 possesses a N-terminal extension of 41 amino acid residues. This extension does not contain a polyproline II helix-binding motif typical of the known eukaryotic parvulins. The *h*Par14 does not accelerate the *cis* to *trans* interconversion of oligopeptides with side chain-phosphorylated Ser(Thr)-Pro moieties as *h*Pin1 did. In contrast, it showed preference of an arginine residue adjacent N-terminal to proline. Northern blot analysis revealed expression of the gene within various human tissues like heart, placenta, liver, kidney and pancreas.

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Key words: Peptidyl-prolyl *cis/trans* isomerase; Human; Parvulin; Pin1; Gene; Sequence

1. Introduction

The third family of peptidyl prolyl *cis/trans* isomerases (PPIases) [1,2], the parvulins, has been established after description of the prototypic enzyme (*E. coli* Par10) found in the *Escherichia coli* cytosol [3]. By a database search, a group of proteins or genes encoding proteins could be identified showing highly significant similarity with the sequence of the catalytic core of parvulin [4,28], establishing HILVK, $(X)_{33-58}$, GGDLGWF, $(X)_{29-31}$, GXHII as common motifs.

Especially some of the prokaryotic members of this group were found to be involved in maturation, transport or assembly of specific proteins [5–11].

Recently, a parvulin homologous protein was identified in human tissue termed Pin1 [12]. This protein shows a strong relationship to a yeast essential protein, PTF1/ESS1 [13–15]. Depletion of the Pin1 or PTF1/ESS1 genes from HeLa cells or

yeast disturbed the cell division by inducing mitotic arrest [12]. Unlike prokaryotic parvulins, Pin1 from human, PTF1/ESS1 from *Saccharomyces cerevisiae*, Dodo from *Drosophila melanogaster* [16], Pin1 from *Aspergillus nidulans* [17], PinA from *Dictyostelium discoideum* and Ssp1 from *Neurospora crassa* [18] share sequence features which N-terminally mediate protein/protein interactions by a WW domain. The WW domain consists of 35–40 amino acid residues. Four conserved aromatic residues (two of which tryptophan) are the dominant characteristic of this sequence motif which was found in a number of unrelated proteins [19,20].

Interaction between Pin1 from human and from *A. nidulans* and many phosphoproteins of the mitotic cycle were shown exclusively in their phosphorylated state [21–23]. This fits the enzymatic properties of all the eukaryotic parvulins mentioned above: Pin1 from human, Ssp1 from *N. crassa* and PTF1/ESS1 from *S. cerevisiae* are highly specific for the oligopeptide substrate with phosphorylated serine or threonine residues preceding proline [15,18,24,25].

The crystal structure of *h*Pin1 complexed with Ala-Pro and a sulfate ion [21] shows a basic cluster composed of two arginine residues (Arg-68, Arg-69) and a lysine residue (Lys-63). This cluster may be responsible for the preferable recognition and catalysis of substrates containing negatively charged amino acid residues preceding proline. The conservation of these residues in the catalytic core of the eukaryotic parvulins becomes obvious by multiple sequence alignment of parvulin sequences.

In this paper we describe the identification, cloning and enzymatic characterization of a human parvulin (*h*Par14), the first eukaryotic member of the parvulin family of PPIases lacking any protein-binding motif supplemented to the catalytic core.

2. Materials and methods

2.1. Database search and cloning of human parvulin

E. coli Par10 (P39159) and *h*Pin1 (*h*Par18) (U49070) were used performing a search for additionally human parvulins in the EMBL GenBank. Two primers were designed for RT-PCR deriving from the obtained clones (hPar1: 5′-TTT GGA TCC ATG CCG CCC AAA GGA AAA AG and hPaar2: 5′-TTT GAA TTC TTA TTT TCT TCC TTC GAC CA). PCR was performed by denaturing the cDNA at 98°C for 1 min, following 25 cycles of amplification (98°C for 15 s, 65°C for 15 s, 74°C for 30 s) and a final extension step at 74°C for 10 min. The PCR product was used screening the human lung cDNA library (λZAPII, Clonetech, Heidelberg, Germany). Plaques were transferred to nitrocellulose filters which were treated with 1.5 M NaCl, 0.5 M NaOH for 1 min, followed with 1.5 M NaCl, 0.5 M Tris-HCl (pH 8.0) for 3 min and finally with 0.2 M Tris-HCl

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Abbreviations: DTT, dithiothreitol; HPLC, high pressure liquid chromatography; IPTG, isopropyl β-D-thiogalactopyranoside; LB, Luria broth; NH-Np, 4-nitroanilide; PCR, polymerase chain reaction; PPIase, peptidyl-prolyl *cis/trans* isomerase; CsA, cyclosporin A

(pH 8.0) for 5 min. Filters were exposed for 2 h to 80°C in a vacuum oven and pre-hybridized at 42°C for 4 h in 0.1 M Pipes buffer pH 7.5 containing 0.8 M NaCl, 5×Denhardt's solution, 100 µg/ml salmon sperm DNA and 50% formamide. Hybridization was performed with [α-^{32}P]dCTP labelled full length human parvulin. After autoradiography, recombinant phages from positive plaques were sequenced.

The database search and alignments were performed using the multiple alignment program packages of Genetyx version 9 (Software Development, Tokyo, Japan). Sequence comparison of various parvulins was performed with the CLUSTALW-program [31].

2.2. Northern Blot analysis

The membrane (Clontech type I membrane (Clontech, Heidelberg, Germany)) was hybridized with the [α-^{32}P]dCTP labelled full length human parvulin for 10 h at 42°C in 50% formamide, 5×Denhardt's solution, 5×SSC, 0.1% SDS and 100 µg/ml salmon sperm DNA. This filter was washed three times with 0.2×SSC containing 0.1% SDS and for 20 min at 62°C. After 3 h, the exposed membrane was analyzed by BAS 1000 (Fuji Film, Tokyo, Japan). A human multiple tissue Northern (MTN) blot was used.

2.3. Expression of human parvulin gene in E. coli

The DNA sequence of the human parvulin gene was amplified using cDNA from human endothelial cells and two primers corresponding to the 5′ and the 3′ region of the gene (5′-GATCGAGCATGCCG-CCCAAAGGAAAAAGTGGTTC-3′ and 5′-GATCGAAAGCTTA-TTTTCTTCCTTCGACCATAAT-3′). The resulting PCR product was purified by agarose gel electrophoresis, digested with *Sph*I/*Hin*dIII and ligated into the vector pQE70 (Qiagen, Hilden, Germany). Restriction endonucleases *Sph*I and *Hin*dIII and T4 ligase were purchased from Boehringer Mannheim and used as recommended by the manufacturers. After transformation into *E. coli*, M15/pREP4 re-

combinants were screened by restriction analysis and controlled by DNA sequencing according to the procedure of the manufacturer. A positive clone designated as hPTTQ was used for overexpression of the recombinant human parvulin.

2.4. Protein purification

Expression cultures were grown in selective 2×YT medium (16 g/l peptone, 10 g/l yeast extract, 5 g/l NaCl) by inoculation of 1 l with 20 ml of an overnight culture of the appropriate strain. After the induction of protein expression at $A_{600} = 0.7$ with 1 mM IPTG, cells were shaken for a further 5 h at 37°C. Cells were harvested by centrifugation at 4°C for 15 min at 6000×g in a Beckmann J2-HC centrifuge. Sedimented cells were resuspended in 2 mM Tris buffer, pH 8.0. Cell rupture was performed by a SLM Aminco French pressure cell. The cell lysate was stirred with 0.1% (v/v) benzonase for 15 min at 4°C and ultracentrifuged in a Beckmann L8 60M centrifuge at 20 000×g for 30 min at 4°C. The supernatant was applied to a Fractogel EMD DEAE-650(M) column (2.5×20 cm), equilibrated with 2 mM Tris buffer, pH 8.0. *h*Par14 passed the column unbounded and was applied to a Fractogel TSK AF-blue column (1×6 cm), equilibrated with 2 mM Tris buffer, pH 8.0. Fractions containing *h*Par14 were obtained by running a linear gradient from 0 to 3 M KCl in 60 ml 2 mM Tris buffer, pH 8.0. The fractions were pooled and dialyzed for 2 h against 3 l of 10 mM HEPES buffer, pH 7.5, containing 0.5 mM DTE. An ion exchange chromatography was performed using a Fractogel EMD SO$_3^-$ 650(M) column (1×6 cm) (Merck, Darmstadt, Germany). The column was equilibrated with 10 mM HEPES buffer, pH 7.5. Protein was applied to the column at a flow rate of 1.5 ml/min. *h*Par14 containing fractions were received by running a linear gradient from 0 to 1 M KCl in 100 ml 35 mM HEPES buffer, pH 7.5. The homogeneity of *h*Par14 was confirmed by SDS-PAGE (silver stained) and RP-HPLC.

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                                            +1
 -30 GCGGTTCAGCGTTCAACAACAAGCTTCCAAGATGCCGCCCAAAGGAAAAAGTGGTTCTGG
                                       M  P  P  K  G  K  S  G  S  G

  29 AAAAGCGGGGAAAGGGGGAGCAGCCTCTGGGAGTGACAGTGCTGACAAGAAGGCTCAAGG
      K  A  G  K  G  G  A  A  S  G  S  D  S  A  D  K  K  A  Q  G

  99 TCCCAAAGGTGGTGGCAATGCAGTAAAGGTCAGACACATTCTATGTGAAAAACATGGCAA
      P  K  G  G  G  N  A  V  K  V  R  H  I  L  C  E  K  H  G  K

 159 AATCATGGAAGCCATGGAAAAAGTTAAAGTCTGGGATGAGATTCAATGAAGTGGCCGCACA
      I  M  E  A  M  E  K  L  K  S  G  M  R  F  N  E  V  A  A  Q

 219 GTATAGTGAAGATAAAGCCAGGCAAGGGGGTGACTTGGGTTGGATGACCAGAGGGTCCAT
      Y  S  E  D  K  A  R  Q  G  G  D  L  G  W  M  T  R  G  S  M

 279 GGTGGGACCATTTCAAGAAGCAGCATTTGCCTTGCCTGTAAGTGGGATGGATAAGCCTGT
      V  G  P  F  Q  E  A  A  F  A  L  P  V  S  G  M  D  K  P  V

 339 GTTTACAGACCCACCGGTTAAGACAAAATTTGGATATCATATTATGATGGTCGAAGGAAG
      F  T  D  P  P  V  K  T  K  F  G  Y  H  I  I  M  V  E  G  R

 399 AAAATAAAATCATATGAAAGACTGAATAAGTTTTATACATTTTGTTTCTTTAAAAGGTAT
      K  *

 459 TACATATTCTTTTGAGCTGGAGCTGCAAGGAAATACAAAAATTTTTAAAAAGAAAAGATA
 519 TTGGATGCTCCTTGTATTCGTTGAAAGCTCTAAGATTGGGTTTGTAGGTGTAAGAGAGGG
 579 TGGGGCTAAGTGAATGTCAACTGTAGTAGGTATTCAGTCAGTCTTTCTCAAAGAGAAGTC
 639 AAGCAGACTCCCTTTAACCTGTATTCTCTTTCCTCCCAGAACTATTTCTAGCTCTCAGTC
 699 TGTCCCATAAATTAATTCAGAACCATCTTCAGGGGAAGCAGATATCAACTCACACTATT
 759 CACACAACTGAAAATTTGGGCATCAAATAGATTAGTGTGTGAGAATCATAAAATAAGTT
 819 CCTAGACAACATTTGTTTTACATGTTAGTCAACTGTGATCTTCCAGGACAGGTGGATTTA
 879 GCTCCACTGTCTTAACATAGAACGTGGCACGTTATGCCTTTCAGTGTTAACTCCTTTCTT
 939 TTTAAATAAATGTTTATTGGAGGAAAAAGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 999 AAAAAAAAAAAAAA
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Fig. 1. Nucleotide sequence of human parvulin. The predicted protein sequence is depicted below. The reading frame of *h*Par14 starts at position +1 of the nucleotide sequence and ends at position 405. An asterisk denotes the stop codon. The oligonucleotide sequences used as probes for Northern blot and PCR are underlined. Accession number of the nucleotide sequence is AB009690 (DDBJ/EMBL/GenBank).

Table 1
Comparison of the *h*Par14 with the homologous protein sequences of other parvulins

	(*)	%Identity	Similarity
H.s. Pin1	(59–163)	34.2	75.8
E.c. Par10	(8–92)	34.5	73.6
S.c. PTF1	(64–173)	20.8	79.2
D.m. Dodo	(62–166)	40.7	75.9
A.n. Pin1	(73–176)	37.0	79.6

(*)The amino acid boundary of the similarity region.
H.s. Pin1 (Q13526) and *H.s.* Par14 (AB009690) from *H. sapiens*, *E.c.* Par10 from *E. coli*, *S.c.* PTF1 (P22696) from *S. cerevisiae*, *D.m.* Dodo (P54353) from *D. melanogaster* and *A.n.* Pin1 (AF0357768) from *A. nidulans*.

2.5. PPIase assay

Measurements were carried out as described previously [15], using Suc-Ala-Xaa-Pro-Phe-NH-Np as substrate. Xaa represents a variable amino acyl residue in the P_1 position of various oligopeptide substrates used for investigation of the substrate specificity. Reported data are given as the mean of three–five measurements. Substrates were purchased from Bachem (Heidelberg, Germany) or prepared according [25]. Stock solutions of various substrates were made in dimethyl sulfoxide.

2.6. Inhibition studies

The substrate Suc-Ala-Arg-Pro-Phe-NH-Np was used in the measurements for inhibition studies. FK506 was a gift from Fujisawa Pharmaceutical, Osaka, Japan. Stock solutions of the inhibitors were prepared in 50% ethanol. The incubation time was 5 min for measurements using FK506 and 15 min for measurements with CsA. Three independent experiments were performed. The PPIase assay without protease was performed according [26] and investigation of the acceleration of refolding the carboxy-methylated RNase T1 variant by *h*Par14 as described in [30].

3. Results and discussion

Genes encoding proteins belonging to the parvulin family of PPIases occur within archaebacteria, eubacteria and eukaryotes like yeast, plants and animals.

An analysis of complete genomes for the occurrence of parvulin-like enzymes seems to be rather arbitrary. However, it becomes quite obvious that the progenitor sequence of 92 amino acids, representing the entire catalytic core, does solely exist in the *E. coli* cytosol. Other parvulins have C-terminal and N-terminal extensions of variable length usually comprising 59–245 amino acids (Fig. 3). Notably, eukaryotic parvulins all have the catalytic core supplemented by a WW domain functioning in protein/protein interactions. A parvulin ap-

proaching the *E. coli* prototypic 10.1 kDa protein in molecular mass has not been identified in eukaryotes until now.

Some of the eukaryotic parvulins were shown to be essential like PTF1/ESS1 in *Saccharomyces cerevisiae* or *h*Pin1 in HeLa cells [12,13]. But striking to the situation in yeast and HeLa cells, a Pin1 related gene which was identified in *Drosophila melanogaster* (*dodo*) was not found to be essential within transgenic flies [4].

A database search was performed to screen for additional parvulin homologous human genes in the EMBL GenBank. Nucleotide sequences from human Pin1 (*h*Par18) and *E. coli* Par10 were used. A number of six clones could be identified by significant homology (AA418536, W81296, AA471033, T65915, AA507634, T65797).

By combination of all received sequence information, a hybridization probe was developed for screening a cDNA library. From a human lung cDNA library, eight clones were obtained. Clone number 1 contained the full length encoding sequence of a gene which was verified by the 5′ upstream 5′-RACE method. The encoding region of the gene consists of 1013 bp and encodes a protein of 131 amino acids (Fig. 1).

A computer similarity search showed the strong relationship of the putative protein to some PPIases of the parvulin family (Table 1). Because of this sequence homology, the encoded protein was assigned to the parvulin family of PPIases and named *h*Par14. The gene was expressed in *E. coli* and the recombinant protein was purified to homology using ion exchange and affinity chromatography. The integrity of the received protein was proved by mass spectrometry and N-terminal sequencing. The experimental molecular mass was determined to be 13 677 Da which is 228 Da lower than the gene-derived protein. However, a N-terminal truncation of two residues (Met-Pro) leads to the close molecular mass of 13 676 Da. This truncated sequence was verified by Edman degradation of the protein.

The PPIase-activity of *h*Par14 was investigated using several methods. Using the conventional chymotrypsin-coupled assay, moderate enzymatic activity could be detected with an array of tetrapeptide substrates (Table 2). This result was confirmed by assays without helper protease [26] and the acceleration of the refolding rate of the denatured decarboxy-methylated RNaseT1 variant by *h*Pin14 [30]. A contamination with *E. coli* PPIases could be excluded by Western blot analysis and the insensitivity of the enzymatic activity toward high concentrations of the cyclophilin and FKBP inhibitors cyclosporin A and FK506. From these experiments it arose that *h*Par14 is

Table 2
Substrate specificity constants (k_{cat}/K_M) of *h*Par14 and comparison of the specificity pattern to related PPIases

Xaa	*h*Par14 k_{cat}/K_M (/M/s)	Relative activity (%)	*E. coli* Par10 Relative activity (%)	SurA Relative activity (%)
Leu	1012	100	100	100
Ala	550	54	40	33
Phe	223	22	74	28
Gln	620	61	24	77
Arg	3950	390	35	N.D
Lys	523	52	14	17
His	137	14	22	27

Substrates were of the type Suc-Ala-Xaa-Pro-Phe-NH-Np: Xaa stands for a variable amino acyl residue. The relative activities were normalized to the value of k_{cat}/K_M of the substrate Suc-Ala-Leu-Pro-Phe-NH-Np. The values for that substrate were estimated as follows: *E. coli* Par10 $k_{cat}/K_M = 1.35/10^7$ M/s and SurA (*E. coli* Par47) $k_{cat}/K_M = 1.9/10^4$ M/s. Measurements were performed in 35 mM HEPES buffer, pH 7.8, at 10°C. The values concerning SurA were calculated from data of [10].
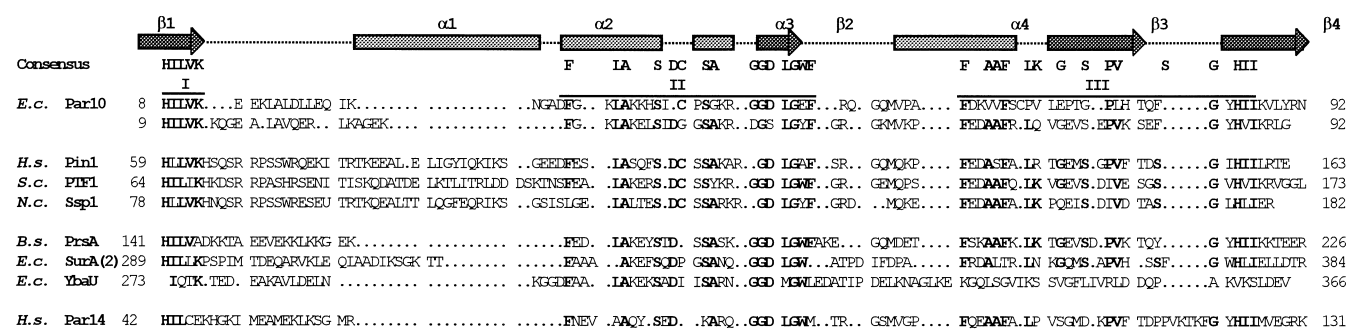
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              β1                      α1                  α2          α3    β2                α4              β3              β4
Consensus     HILVK                            F   IA  S DC  SA   GGD  LGWF          F  AAF  IK  G  S  PV      S    G  HII
              I                                        II                                      III

E.c. Par10  8 HILVK....E EKIALDLLEQ IK....... .......... ..NGADFG.. KIAKKHSI.C PSGKR..GGD LGEF...RQ. GQMVPA.... FDKVVFSCPV LEPTG..PIH TQF......G YHIIKVLYRN  92
            9 HILVK.KQGE A.IAVQER.. LKAGEK.... .......... ......FG.. KIAKELSIDG GSAKR..DGS LGYF..GR.. GKMVKP.... FEDAAFR.LQ VGEVS.EPVK SEF......G YHVIKRLG    92

H.s. Pin1  59 HLLVKHSQSR RPSSWRQEKI TRTKEEAL.E LIGYIQKIKS ..GEEDFES. .IASQFS.DC SSAKAR..GD LGAF..SR.. GQMQKP.... FEDASFA.IR TGEMS.GPVF TDS......G IHIILRTE   163
S.c. PTF1  64 HILIKHKDSR RPASHRSENI TISKQDATDE LKTLITRLDD DSKINSFEA. .IAKERS.DC SSYKR..GGD LGWF..GR.. GEMQPS.... FEDAAFQ.IK VGEVS.DIVE SGS......G VHVIKRVGGL 173
N.c. Ssp1  78 HLLVKHNQSR RPSSWRESEU TRTKQEALIT LQGFEQRIKS ..GSISLGE. .IALTES.DC SSARKR..GD LGYF..GRD. ..MQKE.... FEDAAFA.IK PQEIS.DIVD TAS......G IHLIER     182

B.s. PrsA 141 HILVADKKTA EEVEKKLKKG EK....... .......... ......FED. .IAKEYSTD. SSASK..GGD LGWFAKE... GQMDET.... FSKAAFA.IK TGEVSD.PVK TQY......G YHIIKKTEER 226
E.c. SurA(2) 289 HILIKPSPIM TDEQARVKLE QIAADIKSGK TT....... ......FAAA ..AKEFSQDP GSANQ..GGD LGW...ATPD IFDPA..... FRDALTR.IN KGQMS.APVH .SSF.....G WHLIELLDTR 384
E.c. YbaU 273  IQTK.TED. EAKAVLDEIN .......... .......... ..KGGDFAA. .IAKEKSADI ISARN..GGD MGWLEDATIP DELKNAGLKE KGQLSGVIKS SVGFLIVRLD DQP......A KVKSLDEV   366

H.s. Par14  42 HILCEKHGKI MEAMEKLKSG MR....... .......... ......FNEV .AAQY.SED. .KARQ..GGD LGWM..TR.. GSMVGP.... FQEAAFA.LP VSGMD.KPVF TDPPVKTKFG YHIIMVEGRK 131
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Fig. 2. Sequence alignment of some members of the parvulin family of PPIases. The first line shows the three dimensional structure elements of *h*Pin1 taken from [21]. Bold letters indicate conserved amino acid residues, the corresponding consensus sequence is shown in the second line. The three conserved motifs of the PPIase domain are indicated by underlined I, II and III as shown in the third line. *E.c.* Par10 (P39159), *E.c.* SurA (P21202) and *E.c.* YbaU (P772241) are from *E. coli*, *S.c.* PTF1 (P22696) from *S. cerevisiae*, *H.s.* Pin1 (Q13526) and *H. s.* Par14 (AB009690) from *H. sapiens*, *N.c.* Ssp1 (AJ0006023) from *N. crassa* and *B.s.* PrsA (P24327) from *B. subtilis.*

completely resistant against these inhibitors as could be inferred for a parvulin.

Using the substrate Suc-Ala-Leu-Pro-Phe-NH-Np, the specificity constant k_{cat}/K_M of 1.01/mM/s was determined in the chymotrypsin-coupled assay for *h*Par14. This is about four orders of magnitude lower than the respective value of k_{cat}/K_M for *E. coli* Par10 and about 10-fold less active than SurA from *E. coli* [10] or PrsA from *B. subtilis* (J.-U. Rahfeld, unpublished).

A comparison of the relative values of the specificity constants for various substrates shows a general pattern as it was found for *E. coli* Par10 but a strong preference for a substrate with the basic arginine residue preceding proline (Suc-Ala-Arg-Pro-Phe-NH-Np: k_{cat}/K_M = 3.95/mM/s) (Table 2). In contrast to Pin1 (*h*Par18) but like *E. coli* Par10, *h*Par14 does not accelerate the *cis* to *trans* interconversion of substrates with phosphorylated amino acid residues preceding proline like Ac-Ala-Ala-Ser(PO_3H_2)-Pro-Arg-NH-Np and Ac-Ala-Ala-Thr(PO_3H_2)-Pro-Arg-NH-Np. The lack of recognition of phosphorylated substrates by *h*Par14 underlines the role of the basic cluster in other eukaryotic parvulins for phosphorylated recognition because in *h*Par14 these residues are substituted by Glu-46, Ile-51 and Met-52 (Fig. 2).

Analysis of the sequence of *h*Par14 showed the existence of the sequence motifs typical of the parvulin catalytic core [28] (Fig. 2). Like most other parvulins, *h*Par14 has an N-terminal extension (Fig. 3) but in contrast to *h*Pin1, a WW domain for protein/protein interactions or another known protein-binding

motif could not be identified. Interestingly, using 41 N-terminal amino acid residues of *h*Par14 in a data base search, about a 40–50% homology toward glycine-rich sequences of other proteins like the HMG17 family of non-histone chromosomal proteins [29] and merozoite surface antigenic proteins was obtained. The function of these sequence motifs is still unknown.

A further characteristic feature of *h*Par14 is the absence of a cysteine residue within the motif II (Fig. 2). An involvement of this amino acid residue in the *h*Pin1 catalysis was postulated [21]. Protein variants with a Cys-133 Ala substitution in *h*Pin1 and the corresponding variant Cys-41 Ala of *E. coli* Par10 exhibited enzymatic activities below 1% compared to the wild-type [21,27]. Other prokaryotic parvulins like SurA from *E. coli* and PrsA from *B. subtilis* share this absence of a cysteine within this sequence motif. Their PPIase activities measured using oligopeptide substrates (k_{cat}/K_M = 1–10/mM/ s) is rather small compared to the appropriate values of cyclophilins and FKBPs (k_{cat}/K_M = 0.1–10/µM/s).

In the case of *h*Pin1 it was shown that unspecific substrates lacking the specific phosphorylated side chain cannot activate the full catalytic machinery of the enzyme leading to a > 100-fold reduction of the values of k_{cat}/K_M. The low magnitude of the specificity constant of *h*Par14 of 3.95/mM/s for the most favorable substrate may indicate that the reminiscent of the natural binding partner of this parvulin has not yet been found among the peptides of Table 2. The measured activity seems to represent only the contribution by the desolvatations
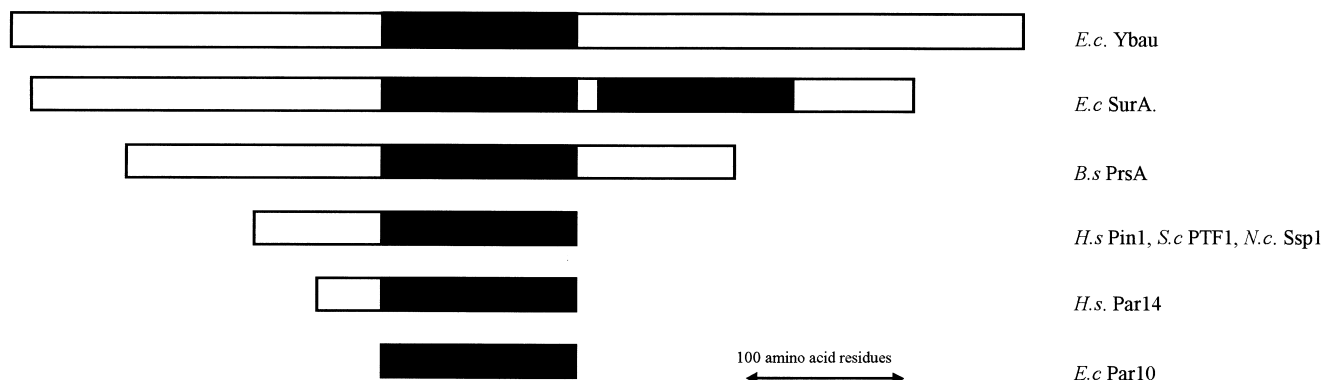

Fig. 3. Comparison of the domain structure of *h*Par14 and some other parvulins. The black colored box indicates the parvulin catalytic core.

A   **B**

β-actin

parvulin

    1 2 3 4 5 6 7 8   1 2 3 4 5 6 7 8

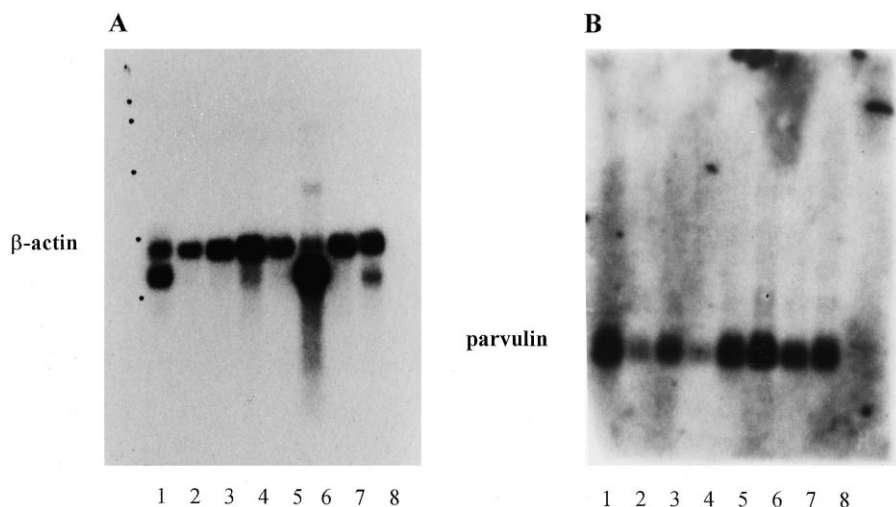Fig. 4. Northern blot hybridization of *h*Par14 probe with poly(A)+RNAs from various human tissues. (A) human β-actin control, (B) human parvulin probe. Lanes 1–8 contain, in order, RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.

mechanism due to the overall hydrophobicity of the substrate-binding pocket of the enzyme without the contribution of specific transition state stabilization of the substrate.

In the case of *h*Par14, it may be hypothesized that the positively charged amino acid in the side chain of the peptide substrate has not yet the correct position.

Northern blot analysis indicated that *h*Par14 is well-expressed in various human tissues (Fig. 4). The presence of a single band of about 1.0 kb was shown (Fig. 4B). Semi-quantitative analysis revealed an overall expression of the gene with a slight reduction in brain and lung compared to heart, placenta, liver, kidney and pancreas (Fig. 4A).

Attempts are made to identify native substrates and interacting proteins to evaluate the physiological function of this PPIase.

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