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DIFFERENTIAL EXPRESSION OF A NOVEL MURINE NON-RECEPTOR PROTEIN TYROSINE PHOSPHATASE DURING DIFFERENTIATION OF P19 EMBRYONAL CARCINOMA CELLS

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SUMMARY: Protein phosphorylation on tyrosine residues is one of the major mechanisms of cell signal transduction and is regulated by protein tyrosine kinases and protein tyrosine phosphatases. Here we report the molecular cloning of an additional member of the protein tyrosine phosphatase-family from differentiated murine P19 embryonal carcinoma cells. This non-receptor protein tyrosine phosphatase, P19-PTP, does not contain regulatory sequences, homologous to the ones found in other non-receptor PTPases. P19-PTP is differentially expressed during *in vitro* differentiation of P19 EC cells, in that P19-PTP mRNA could only be detected in embryoid bodies, derived from P19 cells.

Protein tyrosine phosphorylation is one of the major mechanisms of cellular signaling and is of profound importance for eukaryotic cell proliferation and differentiation. Cellular phospho-tyrosyl levels are regulated by the activities of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPases). Unlike the well characterized protein tyrosine kinases (PTKs [for reviews see refs. 1,2]) relatively little is known about the PTPases. Molecular analysis of the PTPases has resulted in the identification of a common homology domain, containing a perfectly conserved cysteinyl-residue that is absolutely required for their enzymatic activity [3-7]. Homologous PTPase domains have been identified in a number of species, including human, mouse, rat and Drosophila [4,8-10].

The PTPases have been classified in two categories, based on their topology: 1) the cytoplasmically localized non-receptor PTPases and 2) the

ABBREVIATIONS: PTK: protein tyrosine kinase; PTPase: protein tyrosine phosphatase; EC: embryonal carcinoma; RA: retinoic acid; DMSO: dimethyl-sulphoxide; (RT-)PCR: (reverse transcription-) polymerase chain reaction; SDS: sodium dodecyl sulphate; SSC: standard sodium citrate .

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transmembrane receptor-like PTPases [3]. C-terminally of the PTPase-domain a regulatory domain has been identified in several non-receptor PTPases [11-13]. Deletion of this domain *in vitro* by proteolytic cleavage enhances PTPase-activity of these enzymes [11]. The role of cytoplasmic PTPases in cellular proliferation and differentiation is as yet unclear. However, it has been shown that insulin-induced *Xenopus* oocyte maturation is inhibited by micro-injection of purified PTP1B-protein [14], and that PTPase-activity of a 37kD cytoplasmically localized PTPase is enhanced in fibroblasts that are growth arrested by cell contact [15], indicating that PTPases are functional in development and cell proliferation.

While there is clear evidence that PTKs play an important role in early development [16-19], little is known about the possible involvement of PTPases. For that reason, we set out to clone PTPases and study their expression during differentiation of embryonal carcinoma (EC) cells. EC cells [20,21] are widely used as a model system for early murine pre-implantation development, since these pluripotent stem cells can be differentiated *in vitro* and derivatives of all three germ layers have been obtained [22-24].

Here we report the molecular cloning of a novel non-receptor PTPase from the pluripotent murine EC cell line P19. This PTPase, P19-PTP, displays extensive homology to other PTPases in the catalytic domain and is expressed exclusively in embryoid bodies, derived from P19 EC cells.

MATERIALS AND METHODS

Cells: P19 EC cells [25] were cultured as described before [26] in bicarbonate buffered DF medium supplemented with 7.5% foetal calf serum. Differentiation of these cells, growing in monolayer, was done by addition of 10^{-6} M (final concentration) retinoic acid (RA) to the medium. Aggregation of the cells was done by culturing them on a layer of agarose (1%) for 5 days. Subsequently the aggregates were seeded on gelatinized dishes and cultured for another 3 to 6 days. Endoderm-like cells were thus obtained, while neuroectoderm- and mesoderm-like cells were obtained by aggregation and replating in the presence of 10^{-7} M RA and 1% dimethyl-sulphoxide, respectively.

Oligonucleotides and PCR: The degenerative oligonucleotides, used for the polymerase chain reaction are complementary to two highly conserved regions in the PTPase domain (consensus sequences according to [10]) and are depicted in figure 1. Reverse transcription-PCR was done essentially as described before [27]. After reverse transcription, using the 3' oligo (Figure 1) and 30μg of total RNA, PCR was performed for 60 cycles (per cycle 1 min. 95℃, 1 min. 42℃ and 2 min. 72℃), using 2.5u Taq-polymerase (CETUS, USA). The obtained products were fractionated on an agarose gel (1.5%) after digestion with the restriction enzymes EcoRI and BamHI. Subsequently fragments of the appropriate size (120bp) were isolated, ligated into EcoRI/BamHI-opened pBLUESCRIPT SK$^-$ (Stratagene) and sequenced using universal primers and T7-polymerase (Pharmacia).

cDNA-libraries and screening: An oligo(dT)-primed cDNA-library was constructed using RNA from P19 cells, aggregated in the presence of RA (10^{-7}M) for five days and a lambda ZAP cloning kit (Stratagene) exactly as described by the manufacturer. Nitrocellulose filters were hybridized to ^{32}P-labeled probes in 50% formamide - 5xSSC -50mM NaH_2PO_4, pH6.8 - 0.1%SDS - 0.1mg/ml sonicated salmon sperm DNA - 2x Denhardt solution at 42℃ overnight. After hybridization the filters were washed three times with 2xSSC - 0.1% SDS and once with 1xSSC - 0.1% SDS. The ^{32}P-labeled probes were obtained using a multiprime labeling kit (Amersham) routinely using 50 ng DNA fragment and 50μCi [α-^{32}P]dCTP (Amersham). After three rounds of screening the positive clones were picked and subcloned by an *in vivo* excision protocol as described by Stratagene. These subclones were sequenced using universal as well as the degenerative oligos and T7 polymerase (Pharmacia). In addition to this oligo(dT)-primed P19-aggregate cDNA-library, a random as well as oligo(dT)-primed library from murine 3T3-cells (Clontech) was screened as described above.

Northern blotting analysis: RNA, used for Northern blotting analysis, was isolated using a phenol extraction method [28]. Fifteen μg of total RNA was fractionated on a 0.8% formaldehyde-agarose gel and subsequently transferred to nitrocellulose filters. After hybridization to ^{32}P-labeled probes as described above, the filters were washed three times with 2xSSC - 0.1%SDS, once with 0.1xSSC - 0.1%SDS at room temperature and once with preheated (55℃) 0.1xSSC - 0.1%SDS. A 1.4 kb fragment of the rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA [29] was used as a probe for Northern blotting analysis to correct for the amount of RNA loaded per lane.

RESULTS AND DISCUSSION

PTPase-specific probes for hybridization of cDNA libraries were generated by PCR with degenerative oligos, depicted in figure 1, as described in the Materials and methods section. The degenerative oligos are directed against highly conserved stretches in the PTPase-domains [10] and enclose a third highly conserved stretch of amino acids (QGP). Total RNA, isolated from undifferentiated P19 EC cells as well as from *in vitro* differentiated derivatives of P19 cells was pooled and used as a source of RNA for the RT-PCR. Sequence analysis of the 120 bp PCR-products yielded eight different PTPase-clones, containing the

```
5'oligo:
Consensus:                Y   I   N   A   X   F I/M
          5' CC GGATCC TAT ATT AAT GCT III TTT AT 3'
                        C   C   C   C       AC
                            A       A
                                    G
3'oligo:
Consensus:      D   F   W  S/R  M   I   W
          3' CTA AAA ACC GCA TAC TAA ACC CTTAAG GG 5'
              G   G     T G     GG
                        T       T
                        C
```

Figure 1. Degenerative oligonucleotides, directed against conserved sequences in the PTPase-domains, that were used for the polymerase chain reaction. Amino acids are given in single letter code, X being a non-specified amino acid, encoded by three Inosine (I) nucleotides.

```
                                                                        Typ
    PTP3      YINAGFI NMEIPSSSIINQ.....................YIASQGPL PHTCK DFWSMIW  A
    PTP5      YINAGFI DGYQRPSH.........................YIATQGPV HETVY DFWRMIW  B
    PTP31     YINAGFI DGYQRPNH.........................YIATQGPV HETVY DFWSMTW  B
    PTP33     YINAGFI KGVYGPKA.........................YVATQGPF GNTVI DFWSMTW  C
    PTP42     YINAKYI KGVYGPKA.........................YVATQGPL ANTVI DFWSMIW  C
    PTP43     YINAGFI NGYQEKNK.........................FIAAQGPK EETVN DFWRMIW  D
    PTP54     YINAGYA VRWQRVWHSMRLRQRLKGGGRSDDLPLTCLLPSTRVSGAPKEFIATQGPL PNTVG DFWSMIW  E
    PTP59     YINAGFI KGVYGPKA.........................YVATQGPL GNTVI DFWRMTW  C
Consensus     YiNA     g      .........................yi  QGP    T   dFWrMiW
```

Figure 2. Sequence alignment of the polymerase chain reaction-products. Deduced amino acid sequences of the PCR-products, as well as the consensus sequence [10] are given in single letter code. Five different types of PCR-products have been specified A through E.

consensus amino acids QGP between the two degenerative oligos (Figure 2). Two PCR-products exceed the consensus 120 bp in length, PTP3 and PTP54 being 132 and 222 bp, respectively (Figure 2) and we can not exclude that these PCR-products do not encode fragments of PTPases. The eight PCR-products have been divided in five types, members of the same type only differing in one or two amino acids (Figure 2) which may be due to artifacts of the PCR-technique. By sequence-comparison of the PCR-clones with the GenBank/EMBL databases PTP42 was identified to be identical to Receptor Protein Tyrosine Phosphatase α (RPTP α [30]) (also called LCA-Related Phosphatase, LRP [8]). The other PCR-products were homologous but not identical to published PTPases.

In order to obtain full length PTPases, the eight different PCR-products were used as probes to screen a cDNA-library, constructed from RNA of P19 cells, aggregated in the presence of RA. Several λ-phage clones were obtained, subcloned and sequenced. λPTP34 was found to be identical to full length RPTP α [30]. λPTP17, is identical in part to PCR-product PTP33 (Figure 2). Subclones were derived from λPTP17 using the restriction endonuclease sites, depicted in figure 4A, and the complete insert was sequenced. Analysis of the λPTP17-sequence demonstrated that this clone lacks some of the N-terminal conserved amino acids of the PTPase domain (NXXKNRY). In order to obtain full length λPTP17, a random as well as oligo(dT)-primed 3T3 cDNA library was screened with a 600 bp EcoRI/HindIII-probe, located in the extreme 5'part of λPTP17 (Figure 3A). Sequence comparison of λPTP17 and λPTP4.1 demonstrated that λPTP17 lacked an internal fragment of 107 bp, corresponding to basepair positions 151-258 in the combined cDNA sequence (Figure 3C,4).

The combined cDNA of λPTP17 and λPTP4.1 of 2983bp contains an open reading frame, encoding a putative PTPase of 773 amino acids (Figure 4). This PTPase, P19-PTP, schematically represented in figure 3B, contains three in frame

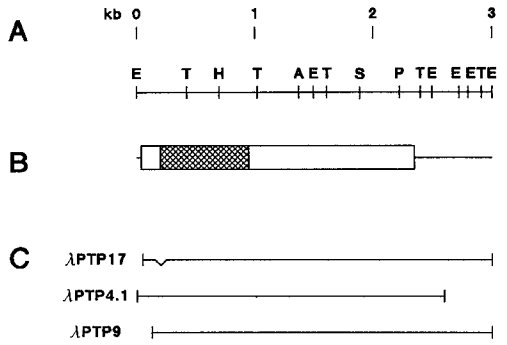

Figure 3. Schematic representation of P19-PTP. (A) Restriction map of P19-PTP, showing all restriction endonuclease sites that were used for subcloning and sequencing (A=ApaI, E=EcoRI, H=HindIII, P=PstI, S=SstI, T=TaqI). (B) Schematic representation of P19-PTP coding and non-coding sequences. Lines indicate non-translated sequences. Boxes represent coding sequences, the hatched box being the PTPase-domain. (C) Alignment of the three lambda phage clones that were used for sequence analysis of P19-PTP. The position of the missing 107 bp in λPTP17 is indicated.

translation initiation sites (Figure 4). No consensus signal sequences or putative transmembrane sequences could be identified in the deduced amino acid sequence of P19-PTP, indicating that P19-PTP belongs to the category of non-receptor PTPases. P19-PTP encodes, like all other non-receptor PTPases [3], only one PTPase domain as indicated in the sequence (Figure 4, 3B) from amino acid position 55 to 299. Sequence comparison of the PTPase-domains of P19-PTP, PTP1B [12,13], TCPTP [11] and the two PTPase domains of RPTP α [30] shows sequence identities ranging from 33 to 36 % between P19-PTP and the other PTPases (Figure 5). In addition, 74 % of the conserved amino acids in the PTPase-domain, as described by Krueger et al. [10], are also conserved in P19-PTP (Figure 5). Notably, the conserved stretch of amino acids, containing the absolutely conserved cysteinyl-residue which has been shown to be essential for PTPase-activity [4-7] (consensus: VVHCSAGVGRTG) is highly conserved in P19-PTP, having only two amino acid substitutions (Figure 5). Besides sequence homology in the PTPase domain, no homology could be found between P19-PTP and any other sequences. It is noteworthy that the regulatory domain, located in the C-terminal part of PTP1B and TC-PTP [3,11-13] is not present in P19-PTP. Several non-receptor PTPases have been identified that, like P19-PTP, lack these regulatory sequences, e.g. STEP [31]. The absence of regulatory domains, similar to the ones found in PTP1B and TCPTP, in our view does not imply that the enzymatic activity of P19-PTP is not regulated. At present the understanding of PTPase-activity regulation is limited. Therefore it can be speculated that P19-PTP contains as yet unidentified regulatory sequences.

P19-PTP

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GAATTCGGGCGGGCGCTGCGGCCGGGCGGG CGGCGGGGGGGACGCGGGAGGATGGAGCAA GTGGAGATCCTGAGGAGGTTCATCCAGAGG   90
                                                    M  E  Q   V  E  I  L  R  R  F  I  Q  R  13
GTCCAGGCCATGAAGAGTCCGGATCACAAT GGGGAGGACAACTTCGCCCGGGACTTCATG CGATTGAGAAGATTGTCTACCAAATATAGA  180
 V  Q  A  M  K  S  P  D  H  N   G  E  D  N  F  A  R  D  F  M   R  L  R  R  L  S  T  K  Y  R  43
ACAGAAAAGATTTATCCCACAGCCACTGGA GAAAAAGAAGAAAATGTTAAAAAGAACAGA TATAAGGACATACTGCCATTTGATCACAGC  270
 T  E  K  I  Y  P  T  A  T  G   E  K  E  E  N  V  K  K  N  R   Y  K  D  I  L  P  F  D  H  S  73
CGAGTTAAGTTGACTTTGAAGACTCCATCC CAAGATTCAGATTATATCAATGCAAATTTT ATTAAGGGTGTGTATGGGCCAAAAGCATAT  360
 R  V  K  L  T  L  K  T  P  S   Q  D  S  D  Y  I  N  A  N  F   I  K  G  V  Y  G  P  K  A  Y 103
GTGGCAACCCAAGGGCCTTTCCGGAATACA GTCATAGACTTCTGGAGGATGATATGGGAG TATAATGTTGTGATGATCGTGATGGCCTGT  450
 V  A  T  Q  G  P  F  R  N  T   V  I  D  F  W  R  M  I  W  E   Y  N  V  V  M  I  V  M  A  C 133
CGAGAATTTGAGATGGGAAGGAAAAAGTGT GAGCGCTACTGGCCTTTGTATGGAGAAGAT CCTATAACATTTGCACCATTTAAAATTTCT  540
 R  E  F  E  M  G  R  K  K  C   E  R  Y  W  P  L  Y  G  E  D   P  I  T  F  A  P  F  K  I  S 163
TGTGAAAATGAACAAGCAAGAACCGACTAC TTCATCCGAACACTTTTACTTGAATTTCAA AATGAATCCCGTCGGCTCTATCAGTTTCAT  630
 C  E  N  E  Q  A  R  T  D  Y   F  I  R  T  L  L  L  E  F  Q   N  E  S  R  R  L  Y  Q  F  H 193
TACGTGAACTGGCCAGACCATGATGTTCCT TCGTCATTTGATTCTATTCTGGACATGATA AGCTTAATGAGGAAATACCAAGAACATGAA  720
 Y  V  N  W  P  D  H  D  V  P   S  S  F  D  S  I  L  D  M  I   S  L  M  R  K  Y  Q  E  H  E 223
GATGTGCCTATTTGTATTCATTGCAGTGCT GGCTGTGGACGAACAGGTGCTATTTGTGCC ATAGATTACACGTGGAACTTACTGAAAGCA  810
 D  V  P  I  C  I  H  C  S  A   G  C  G  R  T  G  A  I  C  A   I  D  Y  T  W  N  L  L  K  A 253
GGGAAAATTCCAGAGGAATTTAATGTATTT AATTTAATACAAGAAATGAGAACACAGAGG CACTCGGCAGTACAAACAAAGGAGCAGTAT  900
 G  K  I  P  E  E  F  N  V  F   N  L  I  Q  E  M  R  T  Q  R   H  S  A  V  Q  T  K  E  Q  Y 283
GAACTTGTTCATAGGGCTATTGCTCAACTG TTTGAAAACAGCTACAACTGTATGAAATTC ATGGAGCACAGAAGATCCGTGATGGTAATG  990
 E  L  V  H  R  A  I  A  Q  L   F  E  N  S  Y  N  C  M  K  F   M  E  H  R  R  S  V  M  V  M 313
AAATTACCACTGGAACTATGGTCAGTTCCA TTGATAGCGAGACGAGACCTCTCCGCCAAA GCCGCCGCGGACTCGAAGTTGCCTTGTAGA 1080
 K  L  P  L  E  L  W  S  V  P   L  I  A  R  R  D  L  S  A  K   A  A  A  D  S  K  L  P  C  R 343
AGGGGATGCCAAGGAAGAAATACTACAGCC ACCAGAACCTCACCCGGTGCCACCCATCCT GACGCCATCACCTCCTTCAGCCTTCCCAAC 1170
 R  G  C  Q  G  R  N  T  T  A   T  R  T  S  P  G  A  T  H  P   D  A  I  T  S  F  S  L  P  N 373
GTTACCACTGTGTGCAGGACAGTGACAGGT ACCACCCAAAGCCAGTGCTGCACATGGCCT CACCAGAGCAACACCCAGCCGACCTCAACA 1260
 V  T  T  V  C  R  T  V  T  G   T  T  Q  S  Q  C  C  T  W  P   H  Q  S  N  T  Q  P  T  S  T 403
GAAGCTATGATAAATCAGCGGACCAATGGG GCAAAAAGTGAATCAGCTATTGAGCACATA GATAAGAAGTTAGAGCGCAATTTAAGTTTT 1350
 E  A  M  I  N  Q  R  T  N  G   A  K  S  E  S  A  I  E  H  I   D  K  K  L  E  R  N  L  S  F 433
GAGATTAAGAAAGTCCCTCTCCAAGAAGGG CCCAAAAGTTTTGATGGGAACACACTCTTG AATAGGGGACATGCGATTAAAATTAAATCT 1440
 E  I  K  K  V  P  L  Q  E  G   P  K  S  F  D  G  N  T  L  L   N  R  G  H  A  I  K  I  K  S 463
GCTTCATCTTCTGTAGTTGACAGAACCTCT AAACCACAGGAGTTAAGTGCAGGTGCCCTA AAGGTTGATGATGTATCTCAGAATTCTTGC 1530
 A  S  S  S  V  V  D  R  T  S   K  P  Q  E  L  S  A  G  A  L   K  V  D  D  V  S  Q  N  S  C 493
GCGGACTGTAGTGCGGCTCATTCACACAGA GCTGCTGAGTCGTCAGAGGAGTCCCAGAGC AACTCACACACACCTCCACGGCCAGACTGC 1620
 A  D  C  S  A  A  H  S  H  R   A  A  E  S  S  E  E  S  Q  S   N  S  H  T  P  P  R  P  D  C 523
TTGCCTCTCGATAAGAAAGGACACGTAACG TGGTCACTTCATGGACCTGAAAATGCCACA CCTGTACCCGACTCACCTGACGGCAAATCC 1710
 L  P  L  D  K  K  G  H  V  T   W  S  L  H  G  P  E  N  A  T   P  V  P  D  S  P  D  G  K  S 553
CCAGATAATCATTCTCAGACTCTGAAAACC GTGAGTTCCACACCCAACTCCACCGCAGAA GAGGAAGCCCACGATCTTACAGAGCACCAC 1800
 P  D  N  H  S  Q  T  L  K  T   V  S  S  T  P  N  S  T  A  E   E  E  A  H  D  L  T  E  H  H 583
AACAGCTCCCCTCTGTTGAAAGCTCCCCTC AGCTTTACCAACCCTCTTCACTCTGACGAC TGGCACTCAGACGGAGGGAGCTCTGATGGT 1890
 N  S  S  P  L  L  K  A  P  L   S  F  T  N  P  L  H  S  D  D   W  H  S  D  G  G  S  S  D  G 613
GCTGTGACCAGGAACAAAACTAGCATTTCA ACAGCAAGTGCCACAGTGTCTCCTGCCAGT AGTGCTGAGAGTGCTTGCCATAGGAGAGTA 1980
 A  V  T  R  N  K  T  S  I  S   T  A  S  A  T  V  S  P  A  S   S  A  E  S  A  C  H  R  R  V 643
TTGCCGATGTCCATTGCCAGACAGGAAGTA GCAGGCACGCCGCATTCAGGTGCTGAGAAA GATGCTGATGTTAGTGAGGAGTCGCCTCCT 2070
 L  P  M  S  I  A  R  Q  E  V   A  G  T  P  H  S  G  A  E  K   D  A  D  V  S  E  E  S  P  P 673
CCTTTACCTGAACGAACTCCTGAGTCTTTT GTATTAGCAGATATGCCTGTAAGACCTGAG TGGCATGAACTTCCAAATCAGGAGTGGTCT 2160
 P  L  P  E  R  T  P  E  S  F   V  L  A  D  M  P  V  R  P  E   W  H  E  L  P  N  Q  E  W  S 703
GAACAAAGGGAATCTGAAGGCTTGACAACC TCTGGAAATGAAAAACATGATGCAGGGGGC ATCCACACAGAGGCTTCTGCAGACTCTCCA 2250
 E  Q  R  E  S  E  G  L  T  T   S  G  N  E  K  H  D  A  G  G   I  H  T  E  A  S  A  D  S  P 733
CCTGCTTTCAGTGACAAGAAAGATCAAATA ACAAAAAGTCCAGCAGAAGTCACAGATATT GGTTTTGGTAATCGCTGTGGAAAACCTAAA 2340
 P  A  F  S  D  K  K  D  Q  I   T  K  S  P  A  E  V  T  D  I   G  F  G  N  R  C  G  K  P  K 763
GGACCAAGAGAGCCACCTTCAGAATGGACA TGATGCAGGGAGTGAAAGGACACTTCACAT TATACTGGAACACTCGAAGTCCACTGAAAG 2430
 G  P  R  E  P  P  S  E  W  T   *                                                            773
CCAGGCTTATAGTATTCCATCTTTAATGTG GGAGCTAGTACGAGTATGATTGTTACCTTA GTGTGAGTTCTTTACCTGCCTCCTTATACT 2520
AACAGGCAAGTAGGTATATAATTTGATAAA GTCCCTAAATATTATTAACAGAAGATGTAA GAATTCTGCATGGTCTAAGTCTTTGTGTAT 2610
CTTACTTGTAAATTATTTGCCCTGAAGTTT TAGAAAACAGTTTCTGAATTTTAAACTTGC TGGATTCATGCAGCCAGCATTGCAGGTTAT 2700
CAGAGATCAAAGATTGTAATAATAATACAT TTTGTAAATTGTAAGCAAAAAGTTATTTTT ATACTATATACTGTCTAATTGTTCATCCTA 2790
ATGGTTCTTGTTTTCATCTAGTCATGGAGA TTCAGTAAGTGCCTTGGAAGAATATTGAAT TCTCTTAGCTCGTGTGTGTTACTTTAAAAT 2880
TTGAATTCAACTGGGGTTAGAAGACTATCA GAATATATGTATGTTTCAGGATATTTGACC TGCCATTAAAAAAACAAACAGTTTTACACT 2970
GCTAAAAAAAAAA                                                                              2989
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Figure 4. cDNA nucleotide and deduced amino acid sequences of P19-PTP. Amino acids are depicted in single letter code. Three in frame translation initiation sites are given in bold type in the nucleotide sequence. The putative PTPase domain is underlined.

P19 EC cells can be differentiated *in vitro* and derivatives of all three germ layers have been obtained [22-24]. Expression of P19-PTP during *in vitro* differentiation of P19 EC cells was investigated by Northern blotting analysis, using a 700bp EcoRI/HindIII P19-PTP-fragment as probe (Figure 3A). P19-PTP-

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P19-PTP    55 KEENVKKNRYKDILHFDHSRVKLTLKTPSQDSDYINANFIKGVYGPKAYVATQGPFRNTVIDFWRMIWEYNVVMIVMACREFEMG
PTP1B      37 LPKNKNRNRYRDVSPFDHSRIKLHQ     EDNDYINASLIKMEEAQRSYILTQGPLPNTCGHFWEMVWEQKSRGVVMLNRVMEKG
TCPTP      39 FPENRNRNRYRDVSPYDHSRVKLQN      AENDYINASLVDIEEAQRSYILTQGPLPNTCCHFWLMVWQQKTKAVVMLNRIVEKE
RPTPα(I) 253 KEENKEKNRYVNILPYDHSRVHLTPVEGVPDSDYINASFINGYQEKNKFIAAQGPKEETVNDFWRMIWEQNTATIVMVTNLKERK
RPTPα(II)546 LPANMKKNRVLQIIPYEFNRVIIPVKRGEENTDYVNASFIDGYRQKDSYIASQGPLLHTIEDFWRMIWEWKSCSIVMLTELEERG
Consensus       N  kNRY     yDhsRV L         dYiNA    g      yi  QGP   T  dFWrMiWeq     vm t   E

P19-PTP   134 RKKCERYWPLYGEDPITFAP  FKISCENEQARTDYFIRTLLLEFQ      NESRRLYQFHYVNWPDHDVPSSFDSILDMISLM
PTP1B     112 SLKCAQYWPQKEEKEMIFEDTNLKLTLISEDIKSYYTVRQLELENL     TTQETREILHFHYTTWPDFGVPESPASFLNFLFKV
TCPTP     114 SVKCAQYWP TDDQEMLFKETGFSVKLLSEDVKSYYTVHLLQLENI     NSGETRTISHFHYTTWPDFGVPESPASFLNFLFKV
RPTPα     332 ECKCAQYWP  DQGCWTYGN  VRVSVEDVTVLVDYTVRKFSIQQVGDVTNRKPQRLITQFHFTSWPDFGVPFTPIGMLKFLKKV
RPTPα     625 QEKCAQYWP  SDGLVSYGD  ITVELKKEEECESYTVRDLLVTNT     RENKSRQIRQFHFHGWPEVGIPSDGKGMINIIAAV
Consensus      KC qYWP         g     v          y  r                R       t WPD gvPe p   l f

P19-PTP   208 RKYQEH  EDVPICIHCSAGCGRTGAICAIDYTWNLLKAGKIPEEFNVFNLIQEMRTQRHSAVQTKEQYELVHRAIAQLFENSYN
PTP1B     190 RESGSLSPEHGPVVVHCSAGIGRSGTFCLADTCLLLMDKRKDPSSVDIKKVLLEMRKFRMGLIQTADQLRFSYLAVIEGAKFIMG
TCPTP     191 RESGSLNPDHGPAVIHCSAGIGRSGTFSLVDTCLVLMEKGDD    INIKQVLLNMRKYRMGLIQTPDQLRFSYMAIIEGAKCIKG
RPTPα(I)  408 KACNPQ  YAGAIVVHCSAGVGRTGTFVVIDAMLDMMHSERK    VDVYGFVSRIRAQRCQMVQTDMQYVFIYQALLEHYLYGDT
RPTPα(II)699 QKQQQQS GNHPITVHCSAGAGRTGTFCALSVLERVKAEGI     LDVFQTVKSLRLQMPHMVQTIFQYEFCYKVVQEYIDAFSD
Consensus             gpivvHCSAGvGRtgt    D  l          v         R qR   vQt  Qy f   a   e      t
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Figure 5. Sequence alignment of the PTPase-domains of P19-PTP, PTP1B, TCPTP and RPTP α (domain I and II). Conserved consensus amino acids are given in bold type.

transcripts were not detected in undifferentiated P19 EC cells, resembling the pluripotent stem cells of the inner cell mass, nor in endoderm- and mesoderm-like cells, obtained by RA-induced differentiation of P19 EC cells in monolayer (Figure 6A). However, upon aggregation of P19 cells, yielding embryoid bodies, P19-PTP is expressed (Figure 6B). As shown in figure 6B, the presence of chemical agents during aggregation of P19 cells, thereby inducing neuronal (in presence of RA) or mesodermal (in presence of DMSO) differentiation has only minor effects on the

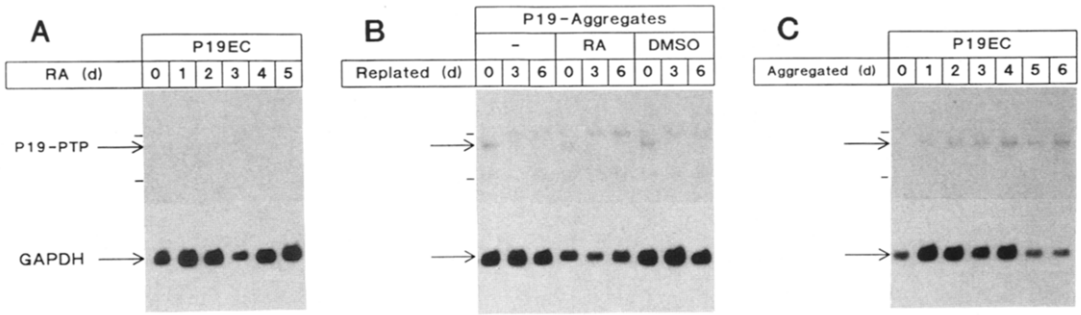

Figure 6. Differential expression of P19-PTP during differentiation of P19 EC cells. (A) P19 EC cells, growing in monolayer were differentiated with RA (10^{-6}M) for the time indicated. Northern blotting analysis was done using 15 μg of total RNA per lane as described in the Materials and Methods section. (B) P19 cells were allowed to aggregate in absence (-) or presence of chemical agents (RA, 10^{-7}M or DMSO, 1%) for five days. Subsequently the aggregates were either replated in tissue culture dishes and cultured for the times indicated or immediately (0d replated) RNA was isolated (C) Time course of aggregation. P19 cells were allowed to aggregate for the times indicated after which RNA was isolated and processed as described under A. The expression of GAPDH was monitored to correct for possible differences in the amount of RNA loaded per lane.

expression of P19-PTP. Replating of the aggregates diminishes P19-PTP expression to undetectable levels (Figure 6B). In order to investigate the kinetics of P19-PTP expression upon aggregation of EC cells, RNA was isolated from P19 cells, aggregated for different periods of time. Figure 6C shows that P19-PTP is expressed constitutively from one day of aggregation onwards. Since P19-PTP is expressed specifically in P19 aggregates it is highly tempting to speculate that P19-PTP exerts a specific function in the aggregates. Further work is required to determine the nature of P19-PTP-functioning in aggregated P19 cells.

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