Increased Proteolytic Processing of Protein Tyrosine Phosphatase μ in Confluent Vascular Endothelial Cells: The Role of PC5, a Member of the Subtilisin Family[†]

Michel Campan,[‡,§] Masao Yoshizumi,[‡,||] Nabil G. Seidah,[⊥] Mu-En Lee,[‡,||,▽] Cesario Bianchi,[*,‡,||,#] and Edgar Haber[*,‡,||]

Cardiovascular Biology Laboratory, Harvard School of Public Health, Department of Medicine, Harvard Medical School, and Cardiovascular Division, Brigham and Women's Hospital, Boston, Massachusetts 02115, and the J. A. De Sève Laboratory of Biochemical Neuroendocrinology, Clinical Research Institute of Montréal, Montréal, Québec, Canada H2W 1R7

Received October 26, 1995; Revised Manuscript Received January 18, 1996[⊗]

ABSTRACT: Cleavage and subsequent release of the extracellular domains of receptor protein tyrosine phosphatases (RPTP) occur at high cell density and may have an important role in regulating their activity. Because cleavage of RPTP occurs at a target motif (RXK/RR) recognized by a family of subtilisin/kexin-like endoproteases, we postulated that members of the subtilisin family may have an important role in this cleavage. We show in this report that the membrane-associated RPTPμ—both in its full 200-kDa form and as a 100-kDa cleavage product—is upregulated 4- and 7-fold, respectively, as human umbilical vein endothelial cells (HUVEC) approach confluence. To determine whether RPTPμ cleavage depended on PC5 (a subtilisin/kexin-like endoprotease present in endothelial cells), we transfected COS cells with expression plasmids coding for RPTPμ and PC5 or the closely related protease PACE4. PC5, but not PACE4, cleaved RPTPμ, and RPTPμ cleavage was absent in COS cells transfected with an expression plasmid encoding a mutant PC5 whose active-site serine had been mutated to alanine. We also performed RNA blot analysis to determine whether PC5 expression was affected by confluence in HUVEC. PC5 mRNA levels were upregulated by more than 30-fold when confluence in HUVEC increased from 25% to 100%. These results indicate that PC5 may have an important role in mediating the cleavage of RPTPμ in response to contact inhibition in HUVEC.

The maintenance of endothelial cells as a monolayer lining the blood vessel lumen depends on the arrest of cell replication when endothelial cells come into contact with one another. This process, often called contact inhibition, is a property of many normal cell types. In endothelial cells, the molecular events regulating contact inhibition have been only partially defined (Mehta et al., 1986; Hai et al., 1989; Bavisotto et al., 1990). In other cell types, protein tyrosine phosphatases (PTP)[1] have been shown to be associated with cell density-dependent inhibition of growth (Pallen et al., 1991; Mansbridge et al., 1992; Rijksen et al., 1993).

Cell growth, differentiation, and proliferation are controlled, in part, by the phosphorylation of tyrosine residues on the numerous proteins that govern intracellular signaling (Cool & Fisher, 1993; Mauro & Dixon, 1994). Tyrosine kinases, which effect phosphorylation, generally stimulate growth, whereas PTP act as growth inhibitors or tumor suppressers (Klarlund, 1985; LaForgia et al., 1991; Brown-Shimer et al., 1992; Woodford-Thomas et al., 1992). PTP have been identified as both cytoplasmic and membrane-associated proteins (Tonks, 1993; Walton & Dixon, 1993). Among the membrane-associated PTP the type II receptor-like PTP (RPTP) family (Fischer et al., 1991), which includes LAR (Streuli et al., 1989), RPTPμ (Gebbink et al., 1991), RPTPδ (Mizuno et al., 1993), RPTPσ (Pan et al., 1993; Yan et al., 1993), and RPTPκ (Jiang et al., 1993), shares sequence and structural homology with cell adhesion molecules. Indeed, RPTPμ (Brady-Kalnay et al., 1993; Gebbink et al., 1993a; Zondag et al., 1995) and RPTPκ (Sap et al., 1994) have been shown to mediate cell−cell aggregation by a homophilic interaction. Since contact inhibition is accompanied by a decrease in tyrosine phosphorylation (Pallen & Tong, 1991; Hunter, 1987) and an increase in membrane-associated PTP activity (Pallen & Tong, 1991), it has been postulated that the homophilic interaction among the extracellular domains of RPTP may signal increased intracellular phosphatase activity and thereby inhibit cell growth (Burgoon et al., 1995).

The extracellular domains of RPTPμ (Brady-Kalnay & Tonks, 1994), LAR (Streuli et al., 1992), RPTPσ (Yan et al., 1993), and RPTPκ (Jiang et al., 1993) can undergo proteolytic cleavage and then be released from the cell surface at high cell density as noncovalently associated proteolytic fragments. Similar proteolytic cleavage of extracellular domains has been demonstrated in the neuron−

[†] Supported in part by a grant from Bristol-Myers Squibb. N.G.S. was supported by a Medical Research Council of Canada Program Grant (PG11474).

* Corresponding authors. Correspondence should be addressed to C.B. at the Molecular Medicine Unit, RW 663, Beth Israel Hospital, 330 Brookline Ave., Boston, MA 02215 (tel: (617) 667-2825. FAX: (617) 667-2913) or to E.H. at Harvard School of Public Health, 677 Huntington Ave., Boston, MA 02115 (tel: (617) 432-1010. FAX: (617) 432-4098).

[‡] Harvard School of Public Health.

[§] Present address: Unité de Recherches de Cardiologie U 8, 33600 Pessac, France.

[||] Harvard Medical School.

[⊥] Clinical Research Institute of Montréal.

[▽] Brigham and Women's Hospital.

[#] Present address: Beth Israel Hospital.

[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1996.

[1] Abbreviations: PTP, protein tyrosine phosphatase(s); RPTP, receptor protein tyrosine phosphatase(s); HUVEC, human umbilical vein endothelial cell(s).

0006-2960/96/0435-3797$12.00/0

glia cell adhesion molecule (Ng-CAM; Burgoon et al., 1995). Because surface expression of proteolytic fragments is restricted to regions of tight cell−cell contact, it has been postulated that contact-induced clustering of RPTP may then lead to increased phosphatase activity at cell−cell contact (Gebbink et al., 1995). However, because proteolytic fragments contain a homotypic binding site, it is also likely that the released RPTP fragments may retain the capacity for homotypic binding and antagonize interactions between RPTP on the surface of adjacent cells (Brady-Kalnay & Tonks, 1995). Despite the potential regulatory effect of these released RPTP fragments on the function of RPTP, the molecular mechanisms mediating their cleavage is unknown. As cleavage of RPTP occurs at a target motif (RXK/RR) (Hosaka et al., 1991) recognized by a family of subtilisin/kexin-like proprotein convertases (also called prohormone convertases) (Roebroek et al., 1994; Seidah et al., 1994), we postulated that members of the subtilisin family may have an important role in this cleavage.

We show in this report that RPTPμ protein expression increases in human umbilical vein endothelial cells (HUVEC) as they approach cell density-dependent inhibition of growth and that proteolytic cleavage of RPTPμ increases simultaneously. To identify the responsible protease, we transfected COS cells overexpressing RPTPμ with cDNA encoding PC5 (Lusson et al., 1993) or PACE4 (Kiefer et al., 1991) convertase. RPTPμ was cleaved by PC5 but not PACE4, and RPTPμ cleavage was absent in COS cells transfected with a PC5 cDNA in which the active-site serine had been mutated to an alanine (Ser354Ala). PC5 expression also increased in a cell density-dependent manner. Our results suggest that PC5 has an important role in the cleavage of RPTPμ when HUVEC reach confluence.

EXPERIMENTAL PROCEDURES

Cell Culture. HUVEC (Clonetics Corp., San Diego, CA) were grown in EGM-UV medium (Clonetics) containing 2% fetal calf serum. Cells were passaged every 4−6 days; cells from the fourth to eighth passages were used in the experiments. The HUVEC were grown to confluence, trypsinized, and seeded at various cell densities by dilution factors ranging from $^1/_{12}$ to $^1/_2$. Cells were collected after two days' growth.

Plasmid Constructs. A full-length cDNA encoding human RPTPμ in the pMT2 expression vector has been described (Gebbink et al., 1993b). Mouse PC5 was cloned into the expression vector pRc/CMV (N. G. Seidah, unpublished data). Human PACE4 cDNA (bp 170−3080) containing the complete translated sequence was inserted between the *Bam*HI and *Eco*RI sites of the expression vector pcDNA3 (InVitrogen).

Mutagenesis. Site-directed mutagenesis was performed by polymerase chain reaction (Higuchi et al., 1988), with the PC5 cDNA in the pRc/CMV vector used as template. For the Ser354Ala mutation, the PC5 catalytic site sequence ACACTGGAACGTCAGCCTCAG was mutated to ACACTGGAACGGCAGCCTCAG with one set of overlapping mismatched primers, PC5M forward (ACACTGGAACGTCAGCCTCAG) and PC5M reverse (TGAGGCTGCCGTTCCAGTG), and one set of external primers, PC5 forward (TGAGAACGGCGTGAGAATG) and PC5 reverse (GGGTGATGTCAGATAGATGG). The mutated PC5 cDNA was then subcloned into the expression vector pRc/CMV and its sequence was confirmed (Sequenase 2.0 kit, United States Biochemical Corp.).

Transient Transfection in COS Cells. Proliferating COS-MT cells were transfected as described (Seed & Aruffo, 1987). Briefly, COS cells at 50%−70% confluence were detached with trypsin and resuspended in Dulbecco's modified Eagle's medium containing 10% serum-Plus (JRH Biosciences), DEAE-dextran (400 μg/mL), chloroquine diphosphate (100 μM), and purified plasmids at various concentrations. After 2 h of incubation at 37 °C the transfection mixture was removed and the cells were treated for 2 min with 10% dimethyl sulfoxide in phosphate-buffered saline for 2 min. The cells were then washed with phosphate-buffered saline and incubated in Dulbecco's modified Eagle's medium containing 5 nM phorbol myristate acetate for 30 min, after which they were incubated in Dulbecco's modified Eagle's medium containing 10% serum-Plus until they were harvested 48 h after transfection.

Western Blot Analysis. Total cell extracts were prepared from HUVEC or COS cells cultured in six-well plates (Falcon). Cells were collected and lysed in Laemmli's buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 1% β-mercaptoethanol, 25% glycerol, and 0.025% bromophenol blue) at an approximate concentration of 0.1×10^6 cells/100 μL of lysis buffer. After 5 min of boiling, 15 μL aliquots were loaded for SDS polyacrylamide gel electrophoresis. Transfer to Immobilon-P membrane (Millipore) was performed with a semidry transfer apparatus (Hoeffer). Western blot membranes were blocked in TBS (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.05% Tween-20) supplemented with 3% bovine serum albumin. Blots were probed with an antiserum (Ab 37, diluted 1/4000) for RPTPμ protein detection (Gebbink et al., 1991) or an antiserum (diluted 1/4000) for PC5 protein detection (Seidah et al., 1994). The blots were washed and incubated with horseradish peroxidase-conjugated donkey anti-rabbit antibody (Amersham) and developed with an enhanced chemiluminescence detection kit (DuPont-NEN). Equal loading was confirmed by staining blots with India ink after Western analysis.

Northern Blot Analysis. Total RNA was prepared from HUVEC with RNAzol-B (Teltest) according to the method of Chomczynski and Sacchi (1987). Northern blots were prepared with 15 μg of total RNA electrophoresed on a 1.3% agarose gel in the presence of 2.2 M formaldehyde and transferred to nitrocellulose Hybond-C extra (Amersham). Hybridization was completed with cDNA probes labeled with ^{32}P-dCTP by random priming (Boehringer Mannheim Corp.) in QuickHyb solution (Stratagene). Blots were washed under high-stringency conditions (0.2 × SSC, 0.1% SDS at 55−60 °C) and exposed to Kodak X-Omat AR film at −70 °C.

RESULTS

Density-Dependent Expression of RPTPμ in Endothelial Cells. Total cell lysates were prepared from HUVEC seeded at various cell densities. Immunoblot analysis of these lysates with polyclonal Ab 37 raised against a C-terminal peptide of the RPTPμ protein (Gebbink et al., 1991) revealed a 200-kDa and a 100-kDa band (Figure 1A). Neither the 200-kDa nor the 100-kDa band was detected by preimmune serum (data not shown). Since the molecular size of RPTPμ is 200 kDa, we inferred that the 100-kDa band represented

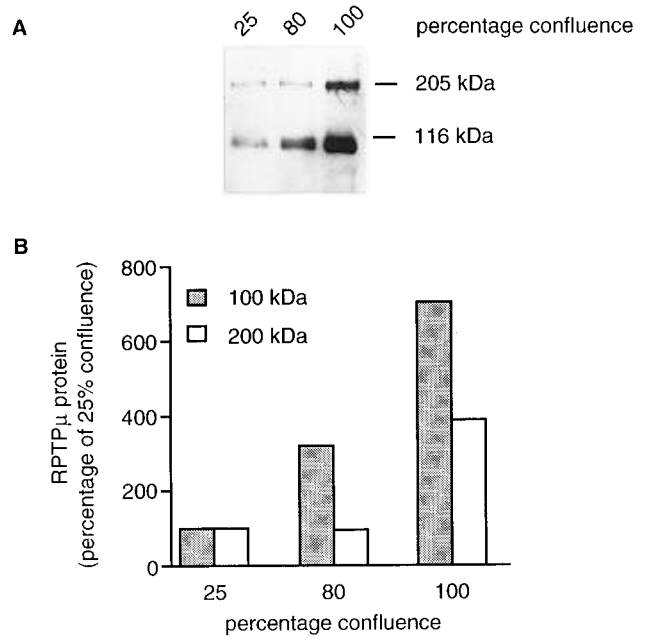

FIGURE 1: Western blot analysis of RPTPμ expression at various cell densities. Confluent HUVEC were detached and seeded at dilutions of $^1/_{12}$, $^1/_6$, and $^1/_2$. After 48 h, cell density was estimated as a percentage of saturating cell density (percentage confluence). (A) Total cell lysates were subjected to 8% SDS gel electrophoresis and immunoblotting with Ab 37 (Gebbink et al., 1991) to detect native and cleaved RPTPμ protein. (B) The intensity (measured by densitometry) of the native (200-kDa) and cleaved (100-kDa) RPTPμ protein bands for each sample was divided by that for the sample prepared at 25% confluence and plotted as a percentage.

a cleavage product. In accord with recent observations of similar cleavage products (Brady-Kalnay, 1994; Gebbink et al., 1995) and consistent with the location of the potential proteolytic cleavage site in the extracellular domain of the RPTPμ protein (Gebbink et al., 1991), this 100-kDa fragment may comprise a segment of the extracellular domain, the transmembrane domain, and the cytoplasmic domain. As HUVEC density increased, the relative amounts of the 200-kDa and the 100-kDa species both increased substantially (Figure 1B), although the amount of 100-kDa protein increased earlier and to a greater degree than did that of the 200-kDa protein. At confluence, the amounts of 100-kDa and 200-kDa RPTPμ species increased by 7- and 4-fold, respectively, when compared with the amounts at 25% confluence. These findings suggest that as cell growth proceeds to confluence expression of RPTPμ and proteolytic cleavage of RPTPμ increase simultaneously.

Proteolytic Processing of RPTPμ Protein by Precursor Convertase PC5 but Not PACE4 in Cotransfection Experiments. RPTPμ contains a potential cleavage site for members of the subtilisin/kexin-like protease family (Gebbink et al., 1991; Hosaka et al., 1991). PC5, a new member of the family, has been shown to be highly expressed in endothelial cells (Beaubien et al., 1995). Also, the P2′ residue in the cleavage of RPTPμ is a lysine, which appears to be well tolerated by PC5 (N. G. Seidah, unpublished observations). Therefore we first determined whether PC5 could cleave RPTPμ. COS cells were cotransfected with an RPTPμ expression plasmid (1 μg/mL) and increasing concentrations of a PC5 expression plasmid (0.001 to 1 μg/mL). In the immunoblot shown in Figure 2A, RPTPμ is undetectable in untransfected COS cells (lane 1) but appears as a doublet at about 200 and 100 kDa after transfection with the RPTPμ plasmid (lanes 2−9). PC5 mRNA is also undetectable in untransfected COS cells (lane 1). As the quantity of PC5 plasmid in the transfection mixture increased with expression of the PC5 protein (lanes 3−9), the concentration of the 100-kDa RPTPμ protein first increased and then decreased (Figure 2B). The intensity of the 200-kDa RPTPμ bands also decreased as expression of PC5 increased (Figure 2A, lanes 7−9). These observations suggest that increasing amounts of PC5 protein first induced increased proteolytic processing of the 200-kDa RPTPμ protein to the 100-kDa species and then effected degradation of both species—perhaps by other proteases—to smaller fragments not visible on the immunoblot.

To confirm that RPTPμ cleavage was specifically mediated by PC5, we generated an inactive PC5 mutant in which the active site serine (Ser 354) (Lusson et al., 1993) was replaced with an alanine. Transfection with the mutant PC5 expression plasmid had no effect on the concentration of the 100-kDa RPTPμ protein (Figure 3, lane 2 versus lane 4), yet transfection with the wild-type PC5 expression plasmid produced an increase in the concentration of the 100-kDa RPTPμ protein (lane 2 versus lane 3). The anti-PC5 immunoblot (Figure 3A) shows that the PC5 mutant and PC5 were expressed equally in this experiment. These results indicate that the increase in the concentration of 100-kDa RPTPμ relative to increasing concentrations of PC5 was attributable to the proteolytic activity of PC5 and not to an artifact of transfection.

The selectivity of PC5 in the cleavage of RPTPμ was examined further by comparing its activity with that of PACE4, the closest PC5 homologue in its family of convertases. COS cells were transfected with both RPTPμ and PACE4 expression plasmids and then examined for expression of the two RPTPμ proteins by immunoblotting (Figure 4A). PACE4 mRNA levels were examined by Northern blotting because an antibody to PACE4 was not available (Figure 4B). The COS cells expressed endogenous PACE4 mRNA (4.4 kb) (lane 1). After transfection a second, 3.2-kb PACE4 mRNA species was visible (lanes 3 and 4). (The PACE4 cDNA product was smaller because the plasmid used in the transfection did not contain parts of the untranslated region.) PACE4 overexpression did not change the concentration of the 200-kDa or the 100-kDa RPTPμ species by immunoblotting (Figure 4A). These observations indicate that PACE4 may have had no effect on proteolytic cleavage of RPTPμ.

Cell Density-Dependent Expression of PC5. After determining that PC5 was capable of cleaving RPTPμ in COS cells, we examined expression of PC5 mRNA in HUVEC at a low (30%) and a high (100%) degree of confluence. PC5 mRNA levels (corrected for loading differences with an 18S oligonucleotide probe) increased by more than 30-fold when confluence grew from 30% to 100% (Figure 5A), and PC5 protein expression increased 4-fold (Figure 5B). In contrast, confluence induced a less than 2-fold increase in furin mRNA (Figure 5C). These observations are consistent with a potential role for PC5, suggested by Figure 1, in the processing of RPTPμ as endothelial cells approach contact inhibition.

DISCUSSION

Inhibition of cell growth at confluence requires that cell−cell contact produce an intracellular signal that ultimately

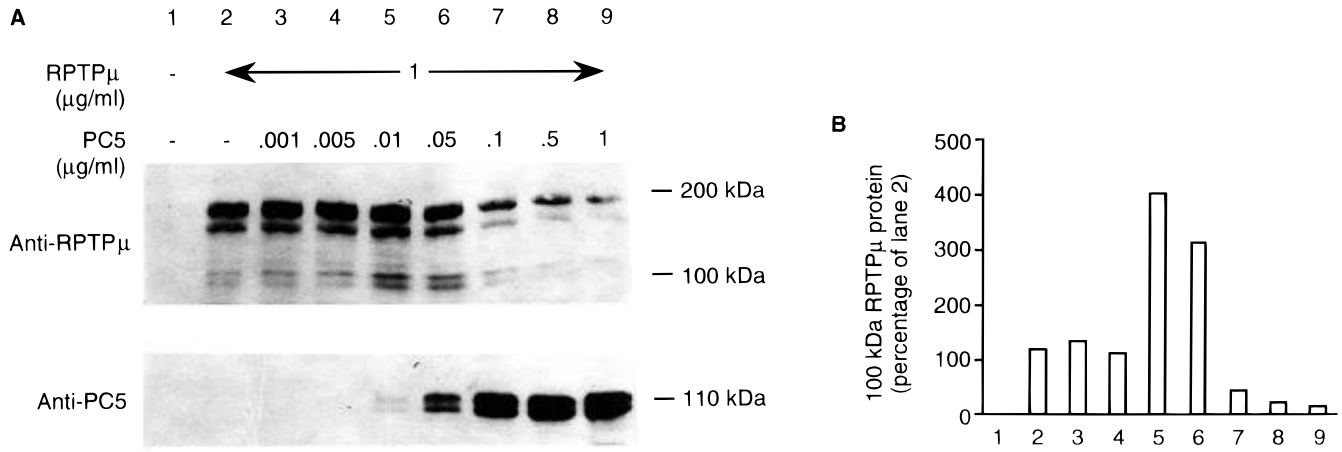

FIGURE 2: Cotransfection assay of proteolytic processing of RPTPμ protein by PC5. COS cells were cotransfected with a constant concentration of RPTPμ cDNA construct (lanes 2−9) and increasing concentrations of PC5 cDNA construct (lanes 3−9). (A) Whole cell extracts were prepared 48 h after transfection and immunoblotted first with polyclonal Ab 37 to detect native and cleaved RPTPμ protein and then with a polyclonal antibody to PC5. (B) Densitometric intensity of 100-kDa RPTPμ protein bands.

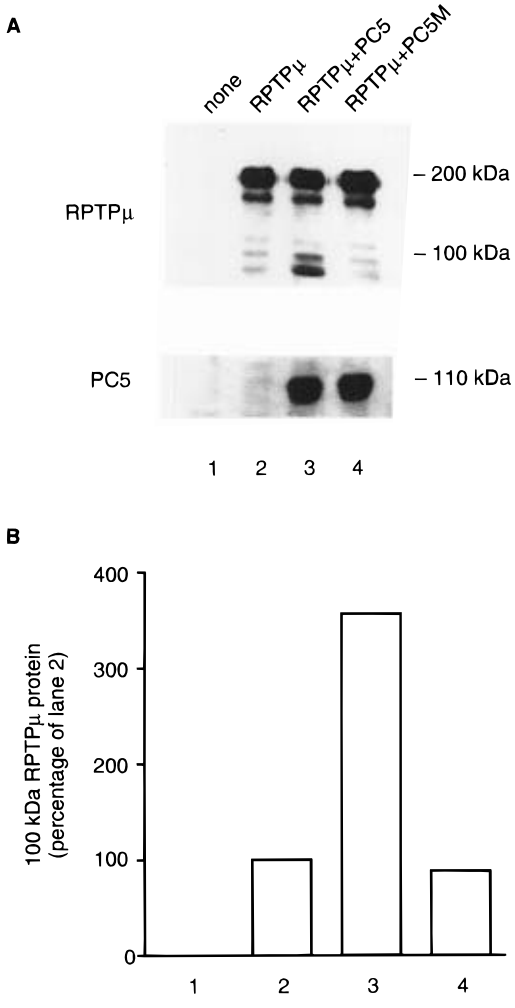

FIGURE 3: Inhibition of RPTP processing by PC5 mutation Ser354Ala. COS cells were transfected (lanes 2−4) or not (lane 1) with RPTPμ cDNA (1 μg/mL) and PC5 cDNA (0.1 μg/mL, lane 3) or PC5M (mutant) cDNA (0.1 μg/mL, lane 4). Transfection and immunoblotting were as described for Figure 2. (A) The transfer membrane was successively probed with antibodies to RPTPμ (Ab 37) and PC5. (B) Densitometric intensity of 100-kDa RPTPμ protein bands.

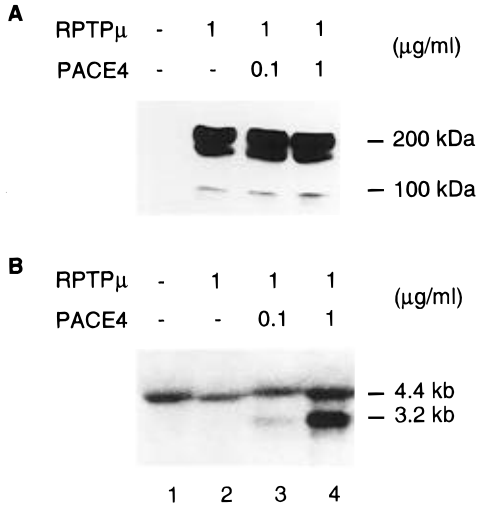

FIGURE 4: Cotransfection assay of PACE4 processing activity in RPTPμ protein. COS cells were cotransfected with RPTPμ and PACE4 cDNA constructs. (A) PACE4 processing activity was measured as described for Figure 1 by immunoblotting with anti-RPTPμ Ab 37. (B) In a parallel experiment, total RNAs were isolated from COS cells transfected under the same conditions and subjected to Northern blot analysis with a radiolabeled PACE4 probe. The probe recognized an endogenous mRNA species (4.4-kb band) and a smaller species corresponding to the expression product of the transfected PACE4 cDNA construct (3.2-kb band).

inhibits DNA replication. Membrane-bound PTP reasonably represent both candidate mediators of the signal and candidate initiators of the cascade that suppresses growth because they have been shown to act as growth inhibitors as well as tumor suppressers. Membrane-bound PTP effect homophilic interactions with their homologues on neighboring cells (Brady-Kalnay et al., 1993; Gebbink et al., 1993a; Sap et al., 1994) that can then result in increased intracellular phosphatase activity and thereby counteract the growth-promoting effects of protein tyrosine kinases (Mooney et al., 1992). For example, in Swiss 3T3 fibroblasts, contact inhibition was associated with an 8-fold increase in total PTP activity (Pallen & Tong, 1991). Likewise, in human embryonic lung fibroblasts, expression of the membrane-bound PTP DEP-1 increased with increasing cell density (Östman et al., 1994). Consistent with this observation, we show here in HUVEC that expression of the membrane-bound phosphatase RPTPμ increased progressively with cell density.

We have also shown that the cell density-dependent increase in RPTPμ protein in HUVEC is accompanied by increasing proteolytic cleavage of the protein. Immuno-

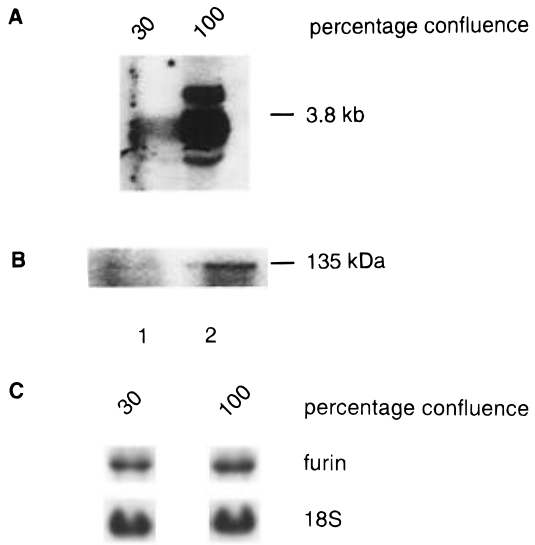

FIGURE 5: Cell density-dependent expression of PC5 and furin in HUVEC. HUVEC were harvested at cell densities estimated as 30% (lane 1) and 100% (lane 2) confluence. (A) PC5 mRNA expression by Northern blot analysis with radiolabeled probe. The blot was also hybridized with an 18S oligonucleotide probe to correct for differences in loading (not shown). (B) PC5 protein expression by Western blot analysis with polyclonal anti-PC5 antibody. (C) Furin mRNA expression by Northern blot analysis with a radiolabeled probe. The blot was also hybridized with an 18S oligonucleotide probe to correct for differences in loading.

reactive RPTPμ protein is expressed as a 200-kDa and a 100-kDa species in HUVEC (Figure 1), as in other cell types (Gebbink et al., 1993a, 1995; Brady-Kalnay & Tonks, 1994; Zondag et al., 1995). In addition, we show here that as cell density increases the relative concentration of the 100-kDa species increases (Figure 1). Similar density-induced increases in proteolytic cleavage have been shown for LAR in HeLa cells (Streuli et al., 1992) and RPTPμ in mink lung cells (Gebbink et al., 1995); however, they have not been shown previously in vascular endothelial cells. Although the outcome of RPTPμ cleavage is not known, it is tempting to speculate that this processing step may have an important role in regulating the function of RPTPμ (Gebbink et al., 1995; Brady-Kalnay & Tonks, 1995).

Although all RPTP appear to undergo proteolytic cleavage, the endoprotease has not been identified. The fact that RPTPμ shares a potential convertase (also called subtilisin/kexin-like proprotein convertase) cleavage site with other receptor-like PTP that include LAR (Streuli et al., 1992), RPTPκ (Jiang et al., 1993), RPTPσ (Yan et al., 1993), and RPTPδ (Mizuno et al., 1993) indicates the importance of the subtilisin family. To find the enzyme that cleaves RPTPμ we studied PC5 (Lusson et al., 1993), a new member of the subtilisin family reported to be well expressed in endothelial cells (Beaubien et al., 1995). After cotransfecting plasmids coding for RPTPμ and PC5 into COS cells, we observed that increasing amounts of 100-kDa RPTPμ coincided with increasing concentrations of PC5 cDNA (from 0.001 to 0.5 μg/mL) (Figure 2). When higher concentrations of PC5 cDNA (0.1 μg/mL or above) were transfected into COS cells, there was a loss of the 200-kDa and the 100-kDa RPTPμ species, suggesting that PC5 is capable of specific proteolytic processing at optimal concentrations and of inducing protein degradation at maximal concentrations. That the increase in concentration of the 100-kDa RPTPμ species did indeed depend on the proteolytic activity of PC5 was demonstrated by our observation that a PC5 mutant in which the active-site serine had been replaced by an alanine did not enhance RPTPμ cleavage (Figure 3). In contrast with PC5, PACE4, its closest homologue among the convertases (Kiefer et al., 1991), did not appear to cleave RPTPμ under conditions in which PC5 was active (Figure 4). In addition to PC5 we found that furin, another member of the convertase family, cleaved RPTPμ (data not shown). This selectivity of different convertases with respect to substrate is consistent with observations about processing of the PTP LAR (Serra-Pages et al., 1994). The P2′ residue corresponding to the LAR convertase cleavage site is an alanine. If the alanine at position P2′ (LAR residue 1154) is mutated to a phenylalanine or an arginine, LAR processing is reduced by at least 20-fold. In contrast with LAR, the equivalent P2′ residue in RPTPμ is a lysine, suggesting that RPTPμ would be cleaved by a convertase different from the one that cleaves LAR.

If PC5 is indeed the convertase responsible for RPTPμ cleavage in HUVEC as they approach confluence, then its expression should increase with increasing cell density. Figure 5 shows that there was a 30-fold increase in the level of PC5 mRNA and a 4-fold increase in PC5 protein production when confluence increased from 30% to 100%—increases consistent with a role for this convertase in the demonstrated cell density-related cleavage of RPTPμ. This marked induction of PC5 mRNA by contact inhibition is in sharp contrast with the less than 2-fold increase in the level of furin mRNA (Figure 5C), which suggests that, even though furin is capable of cleaving RPTPμ, PC5 is the enzyme most likely involved.

In summary, we have shown that cell density-dependent inhibition of growth in HUVEC is associated with an increase in expression of the 200-kDa, membrane-associated protein tyrosine phosphatase RPTPμ and an increase in its proteolytic cleavage to a 100-kDa fragment. This cleavage may depend on the convertase PC5. Increased expression and proteolytic processing of RPTPμ in confluent vascular endothelial cells may have an important role in contact inhibition of this cell type.

ACKNOWLEDGMENT

We thank Dr. Martijn Gebbink of The Netherlands Cancer Institute, Amsterdam, for generously providing Ab 37 and the full-length mouse RPTPμ cDNA in the PMT_2 vector.

REFERENCES

Bavisotto, L. M., Schwartz, S. M., & Heimark, R. L. (1990) *J. Cell Physiol. 143*, 39−51.

Beaubien, G., Schäfer, M. K. H., Weihe, E., Dong, W. J., Chrétien, M, Seidah, N. G., & Day, R. (1995) *Cell Tissue Res. 279*, 539−549.

Brady-Kalnay, S. M., & Tonks, N. K. (1994) *J. Biol. Chem. 269*, 28472−28477.

Brady-Kalnay, S. M., & Tonks, N. K. (1995) *Curr. Opin. Cell Biol. 7*, 650−657.

Brady-Kalnay, S. M., Flint, A. J., & Tonks, N. K. (1993) *J. Cell Biol. 122*, 961−972.

Brown-Shimer, S., Johnson, K. A., Hill. D. E., & Bruskin, A. M. (1992) *Cancer Res. 52*, 478−482.

Burgoon, M. P., Hazan, R. B., Philips, G. R., Crossin, K. L., Edelman, G. M., & Cunningham, B. A. (1995) *J. Cell Biol. 130*, 733−744.

Chomczynski, P., & Sacchi, N. (1987) *Anal. Biochem. 162*, 156−159.

Cool, D. E., & Fisher, E. H. (1993) *Semin. Cell Biol. 4*, 443−453.
Fischer, E. H., Charbonneau, H., & Tonks, N. K. (1991) *Science 253*, 401−406.
Gebbink, M. F. B. G., van Etten, I., Hateboer, G., Suijkerbuijk, R., Beijersbergen, R. L., van Kessel, A. G., & Moolenaar, W. H. (1991) *FEBS Lett. 290*, 123−130.
Gebbink, M. F. B. G., Zondag, G. C. M., Wubbolts, R. W., Beijersbergen, R. L., van Etten, I., & Moolenaar, W. H. (1993a) *J. Biol. Chem. 268*, 16101−16104.
Gebbink, M. F. B. G., Verheijen, M. H. G., Zondag, G. C. M., van Etten, I., Moolenaar, W. H. (1993b) *Biochemistry 32*, 13516−1322.
Gebbink, M. F. B. G., Zondag, G. C. M., Koningstein, G. M., Feiken, E., Wubbolts, R. W., & Moolenaar, W. H. (1995) *J. Cell Biol. 131*, 251−260.
Hai, T. W., Liu, F., Coukos, W. J., & Green, M. R. (1989) *Genes Dev. 3*, 2083−2090.
Higuchi, R., Krummel, B., & Saiki, R. K. (1988) *Nucleic Acids Res. 16*, 7351−7367.
Hosaka, M., Nagahama, M., Kim, W.-S., Watanabe, T., Hatsuzawa, K., Ikemizu, J., Murakami, K., & Nakayama, K. (1991) *J. Biol. Chem. 266*, 12127−12130.
Hunter, T. (1987) *Cell 50*, 823−829.
Jiang, Y. P., Wang, H., D'Eustachio, P., Musacchio, J. M., Schlessinger, J., & Sap, J. (1993) *Mol. Cell. Biol. 13*, 2942−2951.
Kiefer, M. C., Tucker, J. E., Joh, R., Landsberg, K. E., Saltman, D., & Barr, P. J. (1991) *DNA Cell Biol. 10*, 757−769.
Klarlund, J. (1985) *Cell 41*, 707−717.
LaForgia, S., Morse, B., Levy, J., Barnea, G., Cannizaro, L. A., Li, F., Nowell, P. C., Boghosian-Sell, L., Glick, J., Weston, A., Harris, C. C., Drabkin, H., Patterson, D., Croce, C. M., Schlessinger, J., & Huebner, K. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 5036−5040.
Lusson, J., Vieau, D., Hamelin, J., Day, R., Chretien, M., & Seidah, N. G. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 6691−6695.
Mansbridge, J. N., Knuchel, R., Knapp, A. M., & Sutherland, R. M. (1992) *J. Cell Physiol. 151*, 433−442.
Mauro, L. J., & Dixon, J. E. (1994) *Trends Biochem. Sci. 19*, 151−155.
Mehta, P. P., Bertram, J. S., & Loewenstein, W. R. (1986) *Cell 44*, 187−196.
Mizuno, K., Hasegawa, K., Katagiri, T., Ogimoto, M., Ichikawa, T., & Yakura, H. (1993) *Mol. Cell Biol. 13*, 5513−23.
Mooney, R. A., Freund, G. G., Way, B. A., & Bordwell, K. L. (1992) *J. Biol. Chem. 267*, 23443−23446.
Östman, A., Yang, Q., & Tonks, N. K. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 9680−9684.
Pallen, C. J., & Tong, P. H. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 6996−7000.
Pan, M.-G., Rim, C., Lu, K. P., Florio, T., & Stork, P. J. S. (1993) *J. Biol. Chem. 268*, 19284−19291.
Rijksen, G., Völler, M. C. W., & van Zoelen, E. J. J. (1993) *FEBS Lett. 322*, 83−87.
Roebroek, A. J. M., Creemers, J. W. M., Ayoubi, T. A. Y., & Van de Ven, W. J. M. (1994) *Biochimie 76*, 210−216.
Sap, J., Jiang, Y. P., Friedlander, D., Grumet, M., & Schlessinger, J. (1994) *Mol. Cell. Biol. 14*, 1−9.
Seed, B., & Aruffo, A. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 3365−3369.
Seidah, N. G., Chretien, M., & Day, R. (1994) *Biochimie 76*, 197−209.
Serra-Pages, C., Saito, H., & Streuli, M. (1994) *J. Biol. Chem. 269*, 23632−23641.
Streuli, M., Krueger, N. X., Ariniello, P. D., Tang, M., Munro, J. M., Blatter, W. A., Adler, D. A., Disteche, C. M., & Saito, H. (1992) *EMBO J. 11*, 897−907.
Streuli, M., Krueger, N. X., Tsai, A. Y. M., & Saito, H. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 8698−8702.
Tonks, N. K. (1993) *Semin. Cell Biol. 4*, 373−453.
Walton, K. M., & Dixon, J. E. (1993) *Annu. Rev. Biochem. 62*, 101−120.
Woodford-Thomas, T., Rhodes, J. D., & Dixon, J. E. (1992) *J. Cell. Biol. 117*, 401−414.
Yan, H., Grossman, A., Wang, H., D'Eustachio, P., Mossie, K., Musacchio, J. M., Silvennoinen, O., & Schlessinger, J. (1993) *J. Biol. Chem. 268*, 24880−24886.
Zondag, G. C., Koningstein, G. M., Jiang, Y. P., Sap, J., Moolenaar, W. H., & Gebbink, M. F. (1995) *J. Biol. Chem. 270*, 14247−14250.

BI952552D