Abstract. RPTPµ is a transmembrane protein tyrosine phosphatase with an adhesion molecule-like ectodomain. It has recently been shown that RPTPµ mediates homophilic interactions when expressed in insect cells. In this study, we have examined how RPTPµ may function as a cell contact receptor in mink lung epithelial cells, which express RPTPµ endogenously, as well as in transfected 3T3 cells. We find that RPTPµ has a relatively short half-life (3–4 hours) and undergoes posttranslational cleavage into two noncovalently associated subunits, with both cleaved and uncleaved molecules being present on the cell surface (roughly at a 1:1 ratio); shedding of the ectodomain subunit is observed in exponentially growing cells. Immunofluorescence analysis reveals that surface expression of RPTPµ is restricted to regions of tight cell-cell contact. RPTPµ surface expression increases significantly with increasing cell density. This density-induced upregulation of RPTPµ is independent of its catalytic activity and is also observed when transcription is driven by a constitutive promoter, indicating that modulation of RPTPµ surface expression occurs posttranscriptionally. Based on our results, we propose the following model of RPTPµ function: In the absence of cell–cell contact, newly synthesized RPTPµ molecules are rapidly cleared from the cell surface. Cell–cell contact causes RPTPµ to be trapped at the surface through homophilic binding, resulting in accumulation of RPTPµ at intercellular contact regions. This contact-induced clustering of RPTPµ may then lead to tyrosine dephosphorylation of intracellular substrates at cell–cell contacts.

Receptor-like protein tyrosine phosphatases (receptor PTPs) represent a new family of transmembrane proteins that are thought to transduce external signals by dephosphorylating phosphotyrosine residues on intracellular substrates (for review see Walton and Dixon, 1993). Although the receptor PTPs are growing in number and diversity, little is known about the nature of their extracellular ligands and their normal intracellular activities. It is also unknown where at the cell surface receptor PTPs are localized and how delivery and localization to the cell surface is regulated.

We have recently cloned and characterized a mammalian receptor PTP, termed RPTPµ, which has an ectodomain similar to both the Ig-like and fibronectin type III–like domains of cell adhesion molecules (Gebbink et al., 1991). We and others have shown that RPTPµ can mediate cell–cell adhesion when expressed in nonadherent insect cells (Gebbink et al., 1993a; Brady-Kalnay et al., 1993). RPTPµ-mediated cell adhesion is calcium independent and does not require a functional intracellular catalytic domain. Cell–cell adhesion can also be mediated by a closely related receptor PTP, termed RPTPκ (Jiang et al., 1993), but despite their structural resemblance, RPTPµ and RPTPκ fail to undergo heterophilic interactions (Zondag et al., 1995). These results strongly suggest that RPTPµ and related receptor PTPs serve a role in cell–cell recognition and signaling. In general, cell–cell signaling is fundamental to embryonic development, tissue renewal, and tumor suppression. In particular, by coupling cell–cell interaction to the regulation of protein tyrosine phosphorylation, RPTPµ might transduce signals involved in contact-mediated growth inhibition and differentiation. However, experimental data on RPTPµ functioning within a monolayer of mammalian cells are still lacking.

To examine how RPTPµ may function in contact-mediated signaling, we have studied the mechanisms that control the disposition and distribution of RPTPµ at the surface of mammalian cells as a function of cell–cell contact. Through biochemical and immunofluorescence analysis of mink lung epithelial cells, which express RPTPµ endoge-
nously, and 3T3 cells stably transfected with wild-type or mutant RPTPα cDNAs, we show that surface expression of RPTPα increases with cell density and is regulated by cell–cell contact. The results lead to a model in which homophilic binding causes newly synthesized RPTPα to accumulate at regions of cell–cell contact. The cell biological implications of this model are discussed.

Materials and Methods

Cells

Mink lung epithelial cells (CCL64; American Type Culture Collection, Rockville, MD) mouse NIH-3T3 cells (clone 2.2; Livney et al., 1987), monkey COS cells, Rat-1 fibroblasts, human Melfuso melanoma cells, human mammary carcinoma (Lucy), and mouse N1E-115 neuroblastoma cells were cultured in DMEM supplemented with 10% FCS. Porcine aortic endothelial (PAE) cells (Miyazono et al., 1988) were kindly provided by C. H. Heldin (Ludwig Institute, Uppsala, Sweden) and grown in Ham’s F-10 medium (Life Technologies, Inc., Grand Island, NY) with 10% FCS. Rat adrenal pheochromocytoma (PC12) cells were grown in DMEM with 10% FCS and 5% horse serum. Human umbilical vein endothelial cells were isolated by trypsin digestion of umbilical veins and cultured on fibronectin-coated plates in RPMI1640 and M199 media (1:1 mixture) with 20% human serum. SP2/0 myeloma cells and hybridomas were grown in Iscove’s medium supplemented with 10% FCS. Insect SF9 cells were grown in Grace’s medium.

cDNA Constructs

The generation of cDNAs encoding either the full-length human RPTPα or a truncated form (termed either Ex1 or Ex1) lacking both phosphatase domains has been described previously (Gebbink et al., 1993a). A chimeric construct (ExG) containing the extracellular domain of RPTPα fused COOH terminally to glutathione S-transferase (GST) was generated in a two-step polymerase chain reaction procedure. First, GST was amplified using pGEX (Smith and Johnson, 1988) as template using oligonucleotides 5’-GACCTACAGGTTAATAATGATCCCTCCTACTAGGT-3’ and 5’-CCCCCTTAGAT-CAATTTGGAGATGGTGCA-3’. A fragment of RPTPα was amplified with oligonucleotides 5’-CTCAATTGCTACAAGGTGGGCT-3’ and 5’-TAGATAGGGGACATITTAACTGAT-3’. Both overlapping fragments were purified on gel and mixed. Next, a fusion construct was amplified using oligonucleotides 5’-GACCTACAGGTTAATAATGATCCCTCCTACTAGGT-3’ and 5’-CTCAGGCTCCATCAGTCAAGGCT-3’ and recloned as a NotI (bp 1537)-XbaI fragment into a Bluescript vector that contained the remaining NHe-terminal RPTPα coding sequences. The resulting cDNA construct encodes the entire extracellular domain of RPTPα and GST. This construct was subcloned into the eukaryotic expression vector pMT2.

Antibodies

To generate mAbs against the RPTPα ectodomain, we sought to produce large amounts of a secreted ectodomain fusion protein in transfected 3T3 cells, rather than in Escherichia coli. The fusion construct, termed ExG, encodes the entire ectodomain of the human RPTPα molecule (aa 1-742; Gebbink et al., 1991). A chimeric construct (ExG) containing the extracellular domain of RPTPα fused COOH terminally to glutathione S-transferase (GST) was generated in a two-step polymerase chain reaction procedure. First, GST was amplified using pGEX (Smith and Johnson, 1988) as template using oligonucleotides 5’-GACCTACAGGTTAATAATGATCCCTCCTACTAGGT-3’ and 5’-CCCCCTTAGAT-CAATTTGGAGATGGTGCA-3’. A fragment of RPTPα was amplified with oligonucleotides 5’-CTCAATTGCTACAAGGTGGGCT-3’ and 5’-TAGATAGGGGACATITTAACTGAT-3’. Both overlapping fragments were purified on gel and mixed. Next, a fusion construct was amplified using oligonucleotides 5’-GACCTACAGGTTAATAATGATCCCTCCTACTAGGT-3’ and 5’-CTCAGGCTCCATCAGTCAAGGCT-3’ and recloned as a NotI (bp 1537)-XbaI fragment into a Bluescript vector that contained the remaining NHe-terminal RPTPα coding sequences. The resulting cDNA construct encodes the entire extracellular domain of RPTPα and GST. This construct was subcloned into the eukaryotic expression vector pMT2.

D, lanes 2–8; these mAbs also detect a cleavage product of ~100 kD, as will be detailed under Results.

Antiserum 37 (Ab37) raised against a synthetic peptide corresponding to the COOH terminus of RPTPα has been described previously (Gebbink et al., 1991). Mouse mAb 3G4 (Ig2b2) was raised against the extra-cellular fibronectin type III repeats of human RPTPα (Gebbink et al., 1993a). An anti-GST mAb, 2F3, was obtained during the same screen. mAb 10B8, recognizing the extracellular domain of the EGFR receptor, rabbit polyclonal anti-EGFR COOH terminal antisera and anti-PI3Cl (Margolis et al., 1988) were kindly provided by J. Schlessinger (New York University). Anti-diacylglycerol kinase mouse mAbs (Schaap et al., 1993) were kindly provided by D. Schaap (The Netherlands Cancer Institute). For immunoprecipitation of phosphotyrosine-containing proteins, 1 μg antiphosphotyrosine antibody PY20 (Affiniti Research Products Ltd., UK) was used per milligram of total protein. For antiphosphotyrosine immunoblots, antibody 4G10 (Upstate Biotechnology, Inc., Lake Placid, NY) was used.

Transient Expression in COS Cells and Insect SF9 Cells

COS cells were transfected using the calcium phosphate precipitation method described by Gebbink et al. (1991). Insect SF9 cells were infected with recombinant Ac-pVLhFL baculovirus (Gebbink et al., 1993a) for 24 h.

Stable Transfection of 3T3 Cells

Mouse NIH 3T3 cells (clone 2.2, which lacks endogenous EGF receptors; Livney et al., 1987) were cotransfected with the appropriate cDNA construct and a vector with the neomycin resistance gene (pSV2-neo; Spivak et al., 1984) using the calcium phosphate precipitation method. Stable transfecants were selected by G418 resistance (Gibco Life Technologies, Inc.). Single-cell subclones were generated by limited dilution.

Flow Cytometry

3T3 cells were washed with PBS and incubated for 10 min in PBS containing 0.5 mM EDTA and 0.02% (wt/vol) NaN3. Strongly adherent lung cells were collected by incubation for 10 min with trypsin + EDTA containing 0.02% NaN3. Cells were harvested by centrifugation and resuspended; 106 cells were used per staining. FACS® analysis was done by indirect immunofluorescence with fluorescein-conjugated goat anti-mouse F(ab)2 (Zymed Laboratories, Inc., South San Francisco, CA) using a FACScan instrument (Becton Dickinson & Co., Mountain View, CA).

Metabolic Labeling

Cells grown in 10-cm dishes were washed twice with DME and then preincubated for 45 min in methionine-free minimal essential medium. Cells were labeled with 50 μCi/ml [35S]methionine (Amersham International) for 2 h.

Cell Surface Iodination

Cells grown in 10-cm plates were washed three times with PBS and incubated for 15 min in 500 μl PBS with 10 μl lactoperoxidase (100 μg). 10 μl Na125I (1 mCi; Amersham) and 25 μl freshly prepared H2O2 (0.1% in PBS). After 7.5 min of incubation, fresh lactoperoxidase (10 μl) and H2O2 (25 μl) were added. Iodinated cells were lysed and RPTPα precipitated with mAb 3D7 followed by SDS-FAGE analysis and autoradiography.

Immunoprecipitation and Immunoblotting

Cells were washed three times with ice-cold PBS and lysed in NP-40 lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 10% glycerol, 1% NP-40, 2 mM PMSF, 5 μg/ml leupeptin, 2.5 μg/ml aprotinin). After centrifugation, the supernatant was precleared with protein A-Sepharose. The supernatants were precleared with protein A-Sepharose for 30 min. The supernatants were then incubated with the antibody of interest overnight at 4°C. The immune complexes were collected by centrifugation for 15 min at 10,000 × g, washed once with lysis buffer, transferred to a new tube, and washed once with freshly prepared H2O2 (0.1% in PBS). The immune complexes were collected by centrifugation for 15 min at 10,000 × g, washed once with freshly prepared H2O2 (0.1% in PBS). After 7.5 min of incubation, fresh lactoperoxidase (10 μl) and H2O2 (25 μl) were added. Iodinated cells were lysed and RPTPα precipitated with mAb 3D7 followed by SDS-FAGE analysis and autoradiography.
**Figure 1.** Generation and analysis of mAbs against the RPTPμ ectodomain. (A) Schematic representation of the RPTPμ-GST fusion construct (ExG) compared with full-length RPTPμ. MAM, represents the domain homologous to meprin, A5 and μ (Beckmann and Bork, 1993); Ig is the immunoglobulin-like domain; and FNIII is the fibronectin type III-like repeat. The amino acid sequence linking RPTPIx and GST is indicated. (B) SDS-PAGE analysis of ExG protein purified from conditioned medium from ExG-transfected 3T3 cells (Coomassie blue staining). Purified ExG protein was treated with Endo F as indicated. Molecular mass standards are in kilodaltons. (C) Flow cytometric analysis of cell surface expression on untransfected 3T3, stably transfected 3T3[FL], and mink lung cell (MvLu) cultures. Cells were harvested and stained with anti-RPTPl~ mAb (3D7) or control antibody (anti-GST mAb 2F3). (D) SDS-PAGE analysis of anti-RPTPl~ immunoprecipitates. Lysates from 3T3[FL] cells labeled with [35S]methionine for 2 h were precipitated with polyclonal COOH-terminal antipeptide antibody 37 (lane 1), control antibody (anti-GST mAb 2F3; lane C), or with the following anti-RPTPl~ mAbs: lane 2, 4B7 (isotype: IgG2a/K); lane 3, 2G1 (IgG2a/K), lane 4, 2D7 (IgG1/K); lane 5, 3D7 (IgG2a/K); lane 6, 1El (IgG1/K); lane 7, 1D9 (IgG2b/K); and lane 8, 2C10 (IgG2b/K). It is seen that mAbs 2D7 (lane 4) and 1El (lane 6) precipitate RPTPμ less efficiently (probably due to the low affinity of these IgG1 isotypes for protein A, rather than a lower affinity for RPTPμ, since both mAbs work well in FACS® analysis; not shown). mAb 2G1 (lane 3) is seen to be less specific. The mAbs precipitate both the uncleaved form of RPTPμ (200 kD) as well as a cleaved form (100-kD doublet).

**Immunofluorescence Microscopy**

RPTPμ-expressing cells were grown on glass coverslips, fixed in PBS containing 4% paraformaldehyde, and incubated with mAb 3D7 followed by fluorescent staining using FITC-conjugated goat anti-mouse F(ab')2. RPTPμ staining was analyzed using a confocal microscope (model MRC-600; Bio-Rad Laboratories, Richmond, CA).

**Results**

**Endogenous Expression of RPTPμ**

Little is known about the expression pattern of the RPTPμ protein; the highest mRNA levels have been found in murine lung, heart, and brain tissue (Gebbink et al., 1991). As shown in Fig. 2, immunoprecipitation and immunoblot experiments reveal that RPTPμ is expressed in many different cell types, including human and porcine vascular endothelial cells, human mammary carcinoma and melanoma cells, as well as in mink lung epithelial cells and Rat-1 fibroblasts, but not in NIH-3T3 cells, PC12 pheochromocytoma cells, nor in N1E-115 neuroblastoma cells. The highest endogenous expression is observed in mink lung epithelial cells; these cells express about half the levels found in stably transfected NIH-3T3 fibroblasts.

**Biosynthesis and Processing of RPTPμ: Two-subunit Structure and Shedding of the Ectodomain**

Virtually nothing is known about the biosynthesis of RPTPμ. We used mink lung epithelial cells and transfected 3T3 fibroblasts for biosynthesis analysis because these cells show the highest expression levels. As shown in Fig. 3 A, a COOH-terminal antibody precipitates not only the mature 195-kD RPTPμ protein but also a proteolytic product of RPTPμ (100-kD doublet). Similar RPTPμ pro-
surface-iodinated mink cells reveals that both the cleaved and full-length forms of RPTPα are present on the cell surface, at an estimated ratio of approximately 1:1 (Fig. 3 C). A similar ratio of cleaved/uncleaved RPTPα molecules was found in the 3T3 transfectants, as shown below (see Fig. 5 B).

The RPTPα cleavage scheme shown in Fig. 3 B immediately suggests that the NH2-terminal cleavage product, comprising almost the entire ectodomain, may be released into the extracellular environment. To test this possibility, conditioned medium and total cell lysates from transfected 3T3 cells were analyzed by immunoprecipitation. Fig. 3 D shows that, indeed, the 100-kD ectodomain fragment can be immunoprecipitated from the medium of exponentially growing 3T3 transfectants, but not from medium of non-transfected control cells; in total cell lysates, the same antibody precipitates both uncleaved RPTPα and the cleaved ectodomain (Fig. 3 D).

The delivery of newly synthesized RPTPα to the cell surface and its processing was further examined by [35S]methionine pulse-chase analysis in combination with Endo H digestions. During a 30-min labeling pulse, no cleavage is observed (Fig. 4; t = 0). When pulse-labeled cells are subsequently chased for various times, two cleavage products of ~100 kD begin to appear simultaneously. Furthermore, it is seen that newly synthesized RPTPα becomes fully resistant to Endo H treatment, which occurs during intracellular transport through the Golgi apparatus. The proteolytic products are fully Endo H resistant, indicating that cleavage occurs after transit through the Golgi apparatus. It is also seen that at chase periods >2 h, when Endo H–sensitive RPTPα is hardly detectable, not all RPTPα protein has undergone cleavage. It thus appears that both cleaved and uncleaved RPTPα molecules can be transported to the cell surface. We estimate the half-life of RPTPα in 3T3 and mink cells (Fig. 4 B, middle panel) at ~3-4 h; for comparison, the estimated half-life of the EGF receptor in mink cells is >8 h (Fig. 4 B, right panel).

**RPTPα Biosynthesis and Processing Is Independent of Catalytic Activity**

We examined whether the catalytic activity of RPTPα is important for its biosynthesis and delivery to the cell surface. To this end, we performed [35S]methionine pulse-chase analysis of 3T3 cells stably transfected with a truncated, membrane-anchored form of RPTPα (termed ExJ or XJ), which lacks the entire catalytic region. As can be seen in Fig. 4 B (left panel), the biosynthesis of truncated RPTPα, its proteolysis, and estimated half-life are very similar to what is observed with full-length RPTPα. Thus, biosynthesis and intracellular transport of RPTPα do not depend on its catalytic activity.

**Localization of RPTPα at Intercellular Contact Regions and RPTPα Stabilization at the Cell Surface**

Our previous work has shown that RPTPα, when expressed in nonadhesive insect cells, has a uniform distribution along the plasma membrane (Gebbink et al., 1993a). However, the surface localization of RPTPα (or any other receptor PTP) in mammalian cells has not been examined to date.

We analyzed RPTPα expression by immunofluorescence microscopy using mAb 3D7, which is directed against...
the RPTP<sub>μ</sub> ectodomain, in combination with a fluorescence-conjugated second antibody. Fig. 5 A shows that in stably transfected 3T3 cells, intense fluorescence is strictly confined to those regions where the membranes of two cells are directly apposed. A similar restricted localization pattern was observed between adjacent mink lung epithelial cells (results not shown). Note that RPTP<sub>μ</sub> staining is not detected anywhere else on the cell surface, nor in cells that lack physical contact with their neighbors (Fig. 5 A). These observations suggest that RPTP<sub>μ</sub> is trapped and becomes concentrated on the cell surface through homophilic binding initiated by cell–cell contact. Such a mechanism predicts that surface-disposed RPTP<sub>μ</sub>, when trapped through homophilic binding, becomes stabilized and has a prolonged half-life. To test this prediction, we monitored the fate of <sup>125</sup>I-labeled RPTP<sub>μ</sub> at the surface of transfected 3T3 cultures (having numerous cell–cell contacts). Fig. 5 B shows that, indeed, iodinated RPTP<sub>μ</sub> expressed at the surface is stable: even after periods of up to 4 h, the RPTP<sub>μ</sub> ectodomain iodination signal is not significantly attenuated. In <sup>35</sup>S)-methionine pulse–chase experiments carried out at high cell densities, we found a relatively small increase in the estimated half-life of the total population of newly synthesized RPTP<sub>μ</sub> (results not shown). This result is not unexpected in view of RPTP<sub>μ</sub>’s very restricted localization pattern, that is, only at cell contacts; thus, in monolayer cultures the bulk of surface-disposed RPTP<sub>μ</sub> will not undergo homophilic binding and will not become stable.

Another prediction of the very restricted localization of RPTP<sub>μ</sub> is that RPTP<sub>μ</sub> cell surface expression would be upregulated when cell contact increases, that is, at increasing cell density, as tested below.

**RPTP<sub>μ</sub> Surface Expression Is Up-regulated by Cell–cell Contact**

We examined RPTP<sub>μ</sub> surface expression in mink lung epithelial cells as a function of cell density by FACS<sup>®</sup> analysis. Sparse cultures with little or no intercellular contacts show relatively low surface expression (Fig. 6 A, micrograph I and Fig. 6 B). However, when cells are grown to near confluence, RPTP<sub>μ</sub> expression is increased about twofold (Fig. 6 A, micrograph II, and Fig. 6 B). A further increase in cell surface RPTP<sub>μ</sub> levels (about threefold) is observed when cells are highly confluent with maximal intercellular contacts (Fig. 6 A, micrograph III). This density-dependent expression pattern of RPTP<sub>μ</sub> is in marked contrast to that of the receptor for EGF, whose surface expression is significantly downregulated at high cell density (Fig. 6).

The above FACS<sup>®</sup> analysis was complemented and extended by immunoblotting experiments. The results confirm that RPTP<sub>μ</sub> expression is significantly upregulated in high-density cultures, whereas endogenous EGF receptor levels are decreased (Fig. 6). In control experiments, expression of an intracellular signaling enzyme (phospholipase C) is seen to be independent of cell density (Fig. 6 C, left panel).

We also examined cellular phosphotyrosine patterns as a function of cell density. Antiphosphotyrosine immunoprecipitations followed by immunoblotting with antiphosphotyrosine antibody reveal that in high-density cultures, where RPTP<sub>μ</sub> expression is maximal, phosphotyrosine levels are significantly reduced (Fig. 6 C, right panel). The major tyrosine-phosphorylated substrates migrate at an
apparent molecular mass of 120-130 kD, but otherwise remain unidentified. From the same cell lysates, equal amounts of control protein (diacylglycerol kinase) were precipitated, indicating that the differences in RPTPα, EGF receptor, and phosphotyrosine levels are physiological and not due to differences in immunoprecipitation conditions.

Density-dependent Expression of Full-Length and Truncated RPTPα in 3T3 Transfectants

Having shown that surface expression of endogenous RPTPα is upregulated at increasing cell density, we next examined how this regulation occurs. If enhanced RPTPα surface expression would be induced at the transcriptional level, then it should not occur when RPTPα expression is under the control of a constitutive promoter as in stably transfected 3T3 cells. Contrary to this prediction, transfected RPTPα in 3T3 cells shows the same density-dependent expression pattern as endogenous RPTPα in mink cells (Fig. 7). This implies that regulation of surface expression by cell–cell contact occurs posttranscriptionally. Thus, it appears that upregulation of RPTPα surface expression is the direct result of increased cell–cell contact.

A similar density-dependent expression pattern was observed in 3T3 cells transfected with the truncated form of RPTPα (ExJ), which lacks the entire catalytic domain (Fig. 7B, right panel). This demonstrates that contact-induced surface expression of RPTPα is regulated independently of its catalytic activity.

Discussion

Recent studies have shown that RPTPα and a closely related receptor PTP, termed RPTPK, can mediate homophilic but not heterophilic interactions when expressed in non-adhesive insect cells (Gebbink et al., 1993a; Brady-Kalnay et al., 1993; Sap et al., 1994; Zondag et al., 1995). This strongly suggests that these receptor PTPs play a role in cell recognition and subsequent cell–cell signaling. However, although the homophilic binding properties have been assessed in insect cell expression systems, very little experimental data are available on the cell biological aspects of RPTPα function (and that of related receptor PTPs) in mammalian cells.

In this study, we have addressed the possible biological
function of RPTPµ as a cell contact receptor in mink lung epithelial cells and transfected 3T3 fibroblasts. Our major new findings can be summarized as follows: (a) Part of newly synthesized RPTPµ is proteolytically cleaved into two subunits; the NH2-terminal subunit can undergo shedding from the cell surface; (b) RPTPµ has a relatively short half-life; (c) RPTPµ is highly concentrated at regions of close membrane apposition; (d) RPTPµ surface expression increases significantly with increasing cell density (whereas EGF receptor expression shows inverse correlation with cell density); and (e) density-dependent surface expression is regulated at a posttranscriptional level and is independent of RPTPµ catalytic activity. Based on these findings, we propose a model of how RPTPµ surface expression and biological activity is regulated by cell–cell contact, as will be discussed below.

**RPTPµ Biosynthesis and Processing**

During intracellular biosynthesis, RPTPµ can undergo posttranslational cleavage into two subunits, which apparently remain associated in a noncovalent manner. RPTPµ proteolysis was also reported in a recent study by Brady-Kalnay and Tonks (1994). Precisely how the two subunits are cleaved and held together remains to be elucidated. A similar cleavage has been described for the related receptor PTPs LAR and RPTPκ, and also for Ng-CAM (Burgoon et al., 1991; Streuli et al., 1992; Yu et al., 1992; Jiang et al., 1993). Our results indicate, however, that both the full-length and cleaved forms of RPTPµ are present on the cell surface.

It is noteworthy that the NH2-terminal subunit of RPTPµ, which comprises almost the entire ectodomain, is shed from the surface of exponentially growing cells. Shedding of the homophilic binding domain may serve to downregulate the function of RPTPµ as a cell contact receptor. If one assumes that full-length RPTPµ promotes growth inhibition through homophilic binding, then shedding of the ectodomain might be advantageous (and perhaps even necessary) for normal cell growth. An alternative or additional possibility is that the shedded ectodomain may function as a competitive inhibitor of RPTPµ-mediated homophilic interactions and thereby modulate growth behavior. Whatever the precise physiological function, it will be interesting to investigate whether RPTPµ shedding can be induced by growth factors. In this regard, it should be mentioned that shedding of the NH2-terminal domain of another receptor PTP, LAR, is enhanced by protein kinase C-activating phorbol ester (Serra-Pages et al., 1994).

It appears that the half-life of RPTPµ is relatively short (3–4 h) as inferred from [35S]methionine pulse-chase analysis. Furthermore, RPTPµ is rather difficult to radioiodinate in intact cells, suggesting that surface expression levels are relatively low. Taken together, these results support the notion that newly synthesized RPTPµ is rapidly internalized and degraded after being disposed at the cell surface.

**RPTPµ as a Cell Contact Receptor**

The finding that RPTPµ in mammalian cells is strictly localized to regions of intercellular contact (Fig. 5 A), rather than showing a uniform surface distribution as in insect cells (Gebbink et al., 1993a), provides direct support for the view that RPTPµ functions as a cell contact receptor mediating cell-cell signaling. Importantly, the [125I]-labeling experiments of Fig. 5 B indicate that RPTPµ expressed at the cell surface (i.e., the subpopulation concentrated at cell–cell contact regions) is quite stable when compared with the relatively short half-life of the total population of newly synthesized RPTPµ. Furthermore, we find that RPTPµ cell surface expression increases with cell density, that is, when there are more intercellular contacts. This density-dependent expression profile is observed not only in endogenously expressing mink lung epithelial cells but also in transfected 3T3 cells, where RPTPµ transcription is driven by a constitutive promoter. From these results we conclude that density-induced upregulation of RPTPµ surface expression occurs at the posttranscriptional level.

When this article was in preparation, Östman et al.
(1994) reported that expression of a newly cloned receptor PTP (termed DEP-1) is also enhanced with increasing cell density through an unknown mechanism; however, it remains to be seen whether this receptor PTP mediates cell–cell interactions.

The findings discussed here, together with our previous results, support the following model for the regulated expression of RPTPμ (Fig. 8). In sparse cultures without intercellular contacts, RPTPμ is nonfunctional: its surface expression levels are low because of rapid internalization and, furthermore, part of the surface-disposed RPTPμ molecules shed their ectodomain into the medium. Upon cell–cell contact, RPTPμ molecules on apposing cells recognize and bind each other in a homophilic manner, thereby preventing RPTPμ from being internalized. As a consequence, more and more RPTPμ molecules accumulate at regions of cell–cell contact, which then ultimately leads to local clustering of many RPTPμ molecules. Although all our results are consistent with surface-disposed RPTPμ being trapped at the cell surface through homophilic binding, we cannot rule out the possibility that increased RPTPμ biosynthesis may play a role as well.

Whatever the precise underlying mechanism, locally concentrated RPTPμ will promote net tyrosine dephosphorylation of as-yet-undefined substrates at sites of cell–cell contact. It is important to note that in this model, cell–cell contact and subsequent clustering do not affect the basal activity of RPTPμ, which is already very high (at least in vitro; Gebbink et al., 1993b). Instead, contact-induced clustering of RPTPμ is thought to bring the cata-
Figure 7. Regulation of RPTPμ cell surface expression by cell-cell contact in transfected 3T3 cells. Stably transfected 3T3[FL] and 3T3[XJ] cells were grown to low, medium, and high densities, and RPTPμ expression was examined. (A) Cell surface expression as determined by FACS analysis on low (I), medium (II), and high (III) density cell cultures. Cells were stained using anti-RPTPμ mAb 3D7. Numbers represent arbitrary units of fluorescence. (B) Expression of RPTPμ analyzed by immunoprecipitation with mAb 3D7 followed by immunoblotting (mAb 3G4). Left panel, wild-type RPTPμ from 3T3[FL] cells. Right panel, truncated RPTPμ from 3T3[XJ] cells.

lytic domain into proximity with its specific substrates.
This, in turn, will then trigger intracellular signaling. In high-density cultures, we do detect decreased tyrosine phosphorylation of one or more protein(s) in the 110–130-kD region, whose identity is currently unknown (Fig. 6 C). However, it remains to be investigated whether this net tyrosine dephosphorylation is causally related to upregulation of RPTPμ.

In conclusion, we propose that RPTPμ functions as a receptor that signals homophilic cell–cell contact and thereby may regulate, either directly or indirectly, contact-mediated cellular responses such as growth inhibition, differentiation, and/or morphogenesis. As such, RPTPμ and related adhesive receptor PTPs may act in parallel, and perhaps in synergy, with other homophilic cell–cell adhesion molecules like the cadherins. Further understanding of the biological function(s) of RPTPμ awaits the identification and characterization of its physiological substrates.

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