[Frontiers in Bioscience 8, d87-99, January 1, 2003]

RECEPTOR PROTEIN TYROSINE PHOSPHATASES AS MEDIATORS OF CELLULAR ADHESION

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1. ABSTRACT

Receptor protein tyrosine phosphatases (RPTPs) are structurally characterized by the diversity of their extracellular domains (ECDs). These domains display Ig-like, fibronectin type III (FNIII), MAM (meprin, A5, PTPmu), and carbonic anhydrase (CAH) motifs that resemble those present in many cell adhesion molecules (CAMs). However, in contrast to most CAMs, RPTPs also contain an intracellular domain possessing phosphatase activity. This combination makes RPTPs unusual in their ability to directly couple extracellular adhesion mediated events to intracellular signaling pathways. Even though identifying physiologically relevant ligands for RPTPs has proven difficult, recent experiments have shown that RPTPs can bind to themselves (homophilic) as well as to other proteins (heterophilic). For example, the type IIb RPTP, PTPmu?acts as a homophilic cell adhesion protein for epithelial and neural cells while the type V RPTP, PTPbeta/zeta binds a variety of CAMs and ECM components such as N-CAM and pleiotrophin. Interestingly, both PTPmu and PTPbeta/zeta?interact with and regulate the tyrosine phosphorylation level of catenins, which are critical in physiological and pathological events such as cell migration, adhesion and transformation. In addition to their role as CAMs, RPTPs directly interact with intracellular adhesion regulators such as the cadherin/catenin complex, $p130^{cas}$ and GIT1. In summary, RPTPs represent a diverse family of transmembrane proteins that act as adhesion receptors and directly translate this engagement into intracellular signaling by modulating phosphotyrosine levels. Discovering the specific roles of RPTPs as receptors and identifying their ligands may lead to a better understanding of human illnesses whose underlying mechanisms involve cellular adhesion.

2. INTRODUCTION

A variety of human diseases, such as cancer, are the direct result of disruptions in the mechanisms controlling cell division, differentiation and adhesion. In recent years it has been clearly established that tyrosine phosphorylation is one of the critical signaling mechanisms regulating these physiological events. Therefore, understanding the regulatory events responsible for maintaining cellular levels of tyrosine phosphorylation can be of utmost importance in helping us decipher the roots of some human diseases. Tyrosine phosphorylation levels are the result of the balanced action between protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Even though PTKs have been extensively studied, much less is known about their catalytic counterparts, the PTPs. PTPs represent a large and diverse family of proteins catalogued into two large subfamilies (1). The cytoplasmic PTPs represent the majority of phosphatases and as their name indicates are intracellular proteins. The other subfamily, the receptor-like protein tyrosine phosphatases (RPTPs), is composed of a variety of type I transmembrane proteins, characterized by the striking diversity of their extracellular domains (ECDs) (2). Here, we will concentrate on the RPTPs, specifically on their roles as cell adhesion molecules (CAMs) and cell adhesion mediators. The roles of RPTPs during development, axon growth and guidance, cell signaling, and hematopoietic cell differentiation have been comprehensively reviewed in recent review articles covering these topics (3-10).

CAMs have been grouped into four major classes: a) the immunoglobulin superfamily, b) the cadherins, c) the integrins, and d) the selectins (Figure 1) (11). These CAMs mediate both homophilic and heterophilic adhesion and are critical in regulating biological processes such as tissue homeostasis, the immune response, inflammation, axonogenesis, and embryogenesis (12-14). In addition, disruption of these molecules, whether loss or gain of function, results in the onset of diseases such as Pemphigous vulgaris, Leukocyte adhesion deficiency-1 and 2, Glanzmann thrombasthenia, atherosclerosis, diabetic vasculopathy and cancer metastasis (11). RPTPs have been subdivided into five

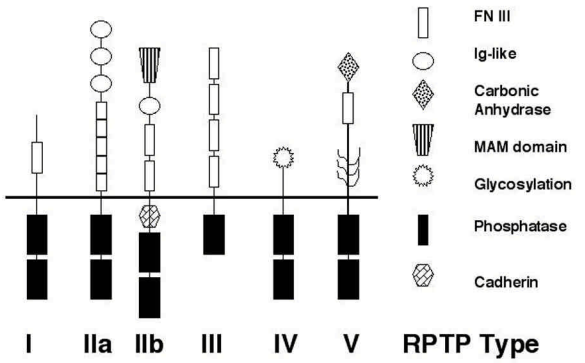

Figure 1. Classification of receptor protein tyrosine phosphatases (RPTPs). RPTPs are classified into 5 types on the basis of their extracellular domains. Diagrams are arranged with the extracellular segments to the top; the thick horizontal line represents the cell membrane. The Type I RTP, CD-45, displays a single FNIII domain. Type II RPTPs are further separated into types IIa and IIb. Both types are characterized by the combination of FNIII domains and Ig-like domains; type IIb also displays an N-terminal MAM domain. Type III RPTPs display only FNIII domains while type IV have short glycosylated extracellular domains. The last type, type V, displays a carbonic anhydrase like domain and a single FNIII domain. The type V RPTP PTPbeta/zeta can also be expressed as a soluble chondroitin sulfate proteoglycan known as phosphacan.

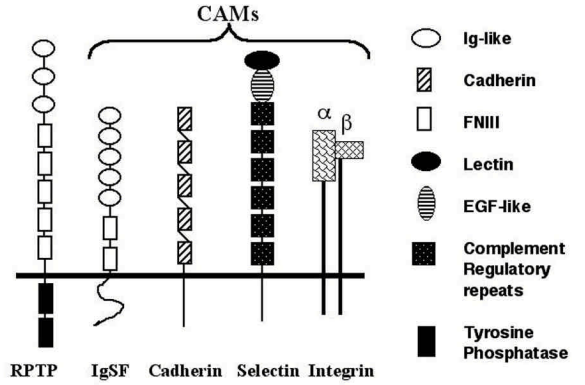

Figure 2. Cell adhesion proteins (CAMs). Diagrams are arranged with the extracellular segments to the top; the thick horizontal line represents the cell membrane. There are four major classes of CAMs. The Ig-superfamily is characterized by having a combination of Ig-like and FNIII domains. Type II RPTPs highly resemble this type of CAM. Members of the cadherin family display variable numbers of cadherin domains bridged by Ca^{2+} ions. Cadherins mediate homophilic, Ca^{2+} dependent cell adhesion. The other two major classes of CAMs are the integrins and the selectins. These CAMs play major roles in the immune system and are heterophilic cell adhesion molecules.

different types based on the structures of their ECDs (7, 15). Of particular interest for the study of cell adhesion are the type II (IIa and IIb), type III and type V RPTPs (Figure 2). The ECDs of type IIa RPTPs are identical to immunoglobulin (Ig) superfamily CAMs in their motif structure, comprising Ig repeats and fibronectin type III repeats (FN III). Type IIb RPTPs exhibit these motifs and, in addition, carry an N-terminal MAM (PTPmu, A5, meprin) domain (15). The ECDs of type III RPTPs consists entirely of various numbers of FNIII repeats (Figure 2). Ig domains are disulfide-bonded structures found in many cell surface receptors and form the homophilic binding sites of cell-cell adhesion molecules such as N-CAM (16). FNIII domains were initially identified on the extracellular cell matrix (ECM) protein fibronectin, where they mediate cell attachment through integrins. Related domains are found on a number of CAMs, where they are involved in a variety of protein-protein binding events (17-19). Finally, type V RPTPs possess a single FNIII domain and an N-terminal CAH-like domain; at least some are synthesized as chondroitin sulfate proteoglycans able to interact with N-CAM and tenascin, suggesting involvement in neuronal adhesion (Figure 2) (20, 21). The presence of functional motifs commonly found on CAMs is consistent with a role of RPTPs in cellular adhesion, whether cell-cell, cell-ECM, or both. While it is important to recognize that most RPTPs are still considered orphan receptors, binding partners for some RPTPs have recently been identified. Here, we discuss the known binding properties and partners of the different RPTP types and their possible roles in human diseases.

3. RPTPs AS HOMOPHILIC AND HETEROPHILIC ADHESION MOLECULES

The study of RPTPs as adhesion molecules is still in its infancy. However, it is already clear that RPTPs are highly specialized CAMs that play a role in both physiological and pathological events such as the development of the nervous system and the transformation of epithelial cells. Therefore, uncovering ligands and signaling pathways for RPTPs may prove critical in developing new therapies for the treatment of human disorders and diseases. Here, we have divided RPTPs by type and will discuss them based on their known adhesive characteristics, whether homophilic or heterophilic.

3.1. Type I RPTPs – CD45

The quest for physiologically relevant "ligands" for RPTPs has been a difficult and largely unfruitful one. In fact, only a few proteins have been identified as possible heterophilic binding partners for the ECDs of RPTPs (6). In most cases it is still unclear whether or not these proteins are physiologically relevant ligands, the binding of which results in changes in phosphatase activity. Binding partners have been identified for type I (CD45), type II (LAR) and type V (PTPbeta/zeta) RPTPs. CD45, the only member of the type I class of RPTPs, is known for its critical role in the development and function of the immune system (8). CD45 was the first RPTP to be identified based on the homology of its phosphatase domain to PTP1B (22). Its ECD displays a single FNIII motif while its intracellular region contains two phosphatase domains (23). CD45 mediates T and B cell receptor activation by regulating the phosphorylation of Src family proteins such as Lyn and Lck (24-27). CD45 knockout mice display disrupted

thymocyte development, and lack Lck and Fyn activation in thymocytes, suggesting involvement in TCR potentiation. Specifically, T cell development was severely affected at the $CD4^-/CD8^-$ to $CD4^+/CD8^+$ transition (28-30). Recently, the beta-galactoside binding protein with broad tissue distribution, Galectin-1 (GAL-1) has been shown to be a ligand for CD45 (31). This interaction involves several GAL-1 molecules binding to one CD45 molecule and is carbohydrate dependent (32). The exact nature and consequence of the interaction is not understood; however, the binding seems to result in lower CD45 and Lyn activity, suggesting direct potential to affect TCR signaling (33). Data suggest that the function of GAL-1 in the immune system involves the mediation of apoptosis in immature and activated T-cells, and that CD45 may modulate this activity (33). However, the exact role played by CD45 in GAL-1 mediated apoptosis remains controversial. Understanding the specific outcome of the interaction between GAL-1 and CD45 on thymocyte activation and/or apoptosis will shed light on both the development and regulation of the immune system. Though is not clear whether other ligands exist, their identification would be a great step for understanding the role of CD45 in the immune system.

3.2. Type II RPTPs

The ECD structures of some RPTPs suggest that they can mediate homophilic cell-cell adhesion, which is characterized by the binding of two cognate molecules across cell membranes. Homophilic cell-cell adhesion proteins have been divided into two major groups: a) the Ig superfamily and b) the cadherin superfamily (34, 35). The ECDs of type II RPTPs comprise Ig-like and FNIII domains, like other members of the Ig superfamily. This observation led scientists to investigate whether type II RPTPs are themselves homophilic cell-cell adhesion proteins. To date, a few type II RPTPs have been shown to be homophilic cell-cell adhesion proteins: PTPmu, PTPkappa, PTPlambda and PTPdelta.?The first RPTP shown to be a homophilic cell adhesion protein was PTPmu(36, 37).?PTPmu is a type IIb RPTP characterized by the presence of one Ig-like domain, four FNIII domains and an N-terminal MAM domain (38). Initial experiments demonstrated that the ECD of PTPmu but not chimeras of the intracellular domain of PTPmu with the ECD of epidermal growth factor receptor (EGFR) mediate cell-cell adhesion. PTPmu mediates cell-cell adhesion in a calcium- and phosphatase domain-independent manner (36, 37). The same observations have now been made for two closely related type IIb RPTPs, PTPkappa and PTPlambda (39, 40). Interestingly, despite their structural similarity (>60% identity at the amino acid level), these three RPTPs fail to undergo heterophilic interactions among themselves (40, 41). Structural and functional studies of the ECD of type IIb RPTPs strongly suggest that the homophilic binding site resides in the Ig-like domain, while the MAM domain functions to discriminate among closely related RPTPs (42). Functional studies of PTPmu demonstrate that it can interact and modulate the phosphorylation of cadherin/catenin complexes (43). PTPmu regulates the function of cadherins by modulating the tyrosine phosphorylation of the complex *in vivo* (44). This makes PTPmu critical in maintaining cell-cell adhesion both through its own ECD and through its regulation of cadherins, which have been shown to play critical roles in neuronal recognition as well as in cellular transformation and metastasis (14, 35, 45, 46). In fact, recent data show that the prostate carcinoma cell line, LnCap, lacks PTPmu expression and that re-expression of PTPmu results in restoration of E-cadherin mediated cell-cell adhesion (47). This observation suggests that in addition to mutations in the catenins, changes in PTPmu expression may be responsible for the downregulation of cadherin function observed in many transformed cells (48). Type II RPTPs are also of importance in the development of the nervous system, particularly in the regulation of axon guidance (6). Recently, the homophilic binding activities of PTPmu2 and PTPkappa have been shown to be involved in the positive regulation of neurite outgrowth *in vitro*. Here again, the interaction with the cadherins seems important since PTPmu can cooperate with N-cadherin to promote neurite outgrowth (49, 50).

Another RPTP that can promote neurite outgrowth is PTPdelta. In contrast to PTPmu, PTPdelta is a type IIa RPTP meaning that its ECD contains Ig-like and FNIII domains but lacks the MAM domain (7). This ECD structure places type IIa RPTPs in the Ig superfamily. PTPdelta is highly expressed in the central nervous system (CNS) and has neural splice variants expressed with both regional and developmental selectivity (51). *In vitro*, PTPdelta is a homophilic cell-cell adhesion molecule able to mediate attractive growth cone guidance for embryonic forebrain neurons (52, 53). Evidence from expression of a putative dominant negative PTPdelta fragment in retinal neurons is consistent with a role for this RPTP in promoting axon growth (54). The phenotype of mutant mice lacking the PTPdelta catalytic domain suggests the involvement of PTPdeltas phosphatase activity in the regulation of hippocampal LTP (55). Generation of complete null mutants for PTPdelta, lacking expression of the ECD, should prove useful in further elucidating the function of this RPTP. Dlar, the closest PTPdelta relative in *Drosophila*, controls axon growth and guidance during development. Dlar null mutants show defects in guidance of motor axons, which either stop growing prematurely, fail to defasciculate from common pathways, or incorrectly bypass target muscles (56). These mutant phenotypes resemble those produced by the over-expression of the CAM fasciclin II on motor neurons, suggesting modulation of cell adhesion mediated events (57). Dlar also appears to act both as a ligand and as a receptor in the guidance of photoreceptors to their targets in the CNS (58). Whether Dlar acts as a homophilic receptor, a heterophilic receptor, or both is unknown. Interestingly, double or triple RPTP mutants in *Drosophila* display either more pronounced errors or reversal of single mutant phenotypes (59). These observations suggest that different RPTPs both cooperate and compete during axon growth and guidance. Altogether, the observed phenotypes argue in favor of these type II RPTPs acting as receptors for cell adhesion and recognition during axonogenesis.

Interestingly, target selection by retinal axons in the *Drosophila* visual system is severely affected by

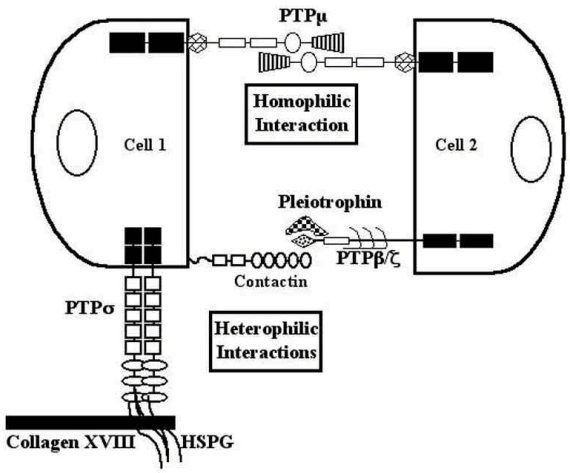

Figure 3. RPTPs mediate both homophilic and heterophilic cell adhesion. The type IIb RPTP, PTPmu, has been shown to mediate homophilic cell adhesion in a manner which resembles the immunoglobulin superfamily in a Ca^{2+} independent fashion. Other RPTPs of these type, such as PTPkappa and PTPlambda also function as homophilic CAMs (top). In contrast, the type V RPTP, PTPbeta/zeta, acts as a heterophilic CAM. PTPbeta/zeta is expressed on glial cells and can modulate neurite outgrowth. It has been shown to interact with pleiotrophin and contactin (middle). Another RPTP able to mediate heterophilic adhesion is PTPsigma which has been shown to interact with heparan sulfate proteoglycans like collagen XVIII and agrin. This interaction is dependent on the presence of heparan sulfate side chains (bottom).

mutations in N-cadherin as well as those in type II RPTPs (PTP69D). *Drosophila* photoreceptor cells (R cells) project either to the lamina or the medulla in the optic lobe. R1-R6 axons project to the lamina while R7 and R8 project to separate layers of the medulla. In *PTP69D* mutants, R1-R6 axons erroneously project through the lamina and terminate in the medulla, while in N-Cadherin mutants, these axons fail to defasciculate and to reach their targets in the lamina. In addition, R7 axons in N-cadherin mutants mistarget to the R8 layer within the medulla. These data show that both cadherins and RPTPs are involved in target selection and recognition by retinal axons and further support a close relationship between these two complex families of adhesion proteins (60, 61).

We have discussed the type II RPTPs as homophilic cell-cell adhesion molecules. In addition, two other type IIa RPTPs, LAR and PTPsigma, have been shown to act as heterophilic receptors, and both appear to be involved in axon growth and guidance (62). To date, no ligand has been identified for PTPsigma, but initial studies suggest that PTPsigma binds to heparan sulfate proteoglycans (HSPGs) present in embryonic retina and glial endfeet (63). The interaction between the PTPsigma ECD and HSPGs is dependent on the presence of heparan sulfate side chains (63). Site-directed mutagenesis studies have identified the first Ig-like domain of PTPsigma as required for binding HSPGs; however, it is not known if binding to these HSPGs results in changes in intracellular catalytic activity. PTPsigma is strongly expressed within chicken retinal and tectal axons where interactions with its putative ligand(s) appear crucial for axon growth and growth cone structure. Blocking ligand binding through the use of PTPsigma antibodies or a soluble form of the PTPsigma ECD results in reduced axon growth, suggesting that ligand binding to PTPsigma is a positive signal for axon growth (64, 65). Recently, retroviral expression of the PTPsigma ECD has been used to block endogenous PTPsigma-ligand sites *in ovo*. Results suggest that PTPsigma function is necessary for sustained growth and correct termination of retinal axons in the optic tectum, and again suggest that ligand binding positively regulates axon growth (66). In addition, expression of a putative dominant negative form of PTPsigma enhances neurite growth *in vitro* (66), suggesting that the PTPase activity of PTPsigma is inhibitory to axon growth. Together, the data suggest a model in which ligand binding to PTPsigma positively regulates axon growth because it relieves a neurite growth inhibitory signal by downregulating PTPase activity.

Two potential physiologically relevant ligand HSPGs whose expression overlaps that of PTPsigma during retinal development are agrin and collagen XVIII (Figure 3) (63). The specific interactions of PTPsigma and these ECM proteins are currently under study. *In vivo*, anatomical and histological analysis of PTPsigma$^{-/-}$ animals showed a decrease in overall brain size with a severe depletion of luteinizing hormone-releasing hormone-immunoreactive cells in the hypothalamus and hypomyelination of peripheral nerves. Although the mechanisms involved in these defects are unknown, the expression pattern of PTPsigma, its *in vitro* effects on neurite outgrowth, and its ability to bind HSPGs suggest that it could function as an axon growth-regulatory receptor during axonogenesis.

The most extensively studied type II RPTP is LAR. LAR structure highly resembles that of the other vertebrate type IIa RPTPs, PTPsigma and PTPdelta. In fact, it is believed that all three arose from a common ancestral gene through gene duplication (67). Like PTPsigma, LAR can act as a heterophilic CAM. Available data suggest that one ligand for LAR is the laminin-nidogen complex (68). Nidogen-1 is a major mediator in the formation of ternary complexes with laminins, collagen IV, perlecan and fibulin. Alternative splicing of a small exon within the fifth FNIII domain of LAR regulates binding to the laminin-nidogen complex, such that inclusion of this exon results in disruption of the binding activity (68). This observation suggests that alternative splicing, a common feature in LAR and other type II RPTPs, regulates ligand selection and specificity (67). Interestingly, laminin subunits, together with nidogen-1, have been identified within the basement membrane of epithelial ducts and alveoli in mouse mammary gland, which shows impaired terminal differentiation at late pregnancy in LAR$^{-/-}$ mice (69, 70). Interactions and signaling mediated by laminin-nidogen binding to the LAR ECD may regulate milk production by the mammary epithelium. LAR is also required for appropriate regeneration of adult peripheral nerve (71). Thus LAR, like its relatives in the fly, may function in developmental axon guidance.

In summary, the type II RPTPs represent an exciting group of Ig superfamily members with the potential to directly couple extracellular events to intracellular signal transduction pathways regulated by tyrosine phosphorylation. As is the case with other CAMs, changes in the expression pattern of these RPTPs can produce significant abnormalities that may aid in the onset of cellular transformation or result in defective axonal projections.

3.3 Type V RPTPs

Type V RPTPs are characterized by the unusual presence of an extracellular CAH-like domain thought to function as a hydrophobic binding pocket for a heterophilic ligand (72). The two members of this RPTP class, PTPbeta/zeta and PTPgamma? can be expressed as either long or short forms. The two forms are distinguished by an 860 amino acid stretch in the "spacer region", the region between the CAH domain and the FNIII domain. In addition, PTPbeta/zeta can be expressed as a chondroitin sulfate proteoglycan, and its ECD can be secreted independently from the rest of the protein to form part of the ECM (73, 74). This secreted domain, known as phosphacan, is strongly expressed by mature glial cells suggesting that its expression is regulated during glial differentiation (75). In contrast, the long and short forms of PTPbeta/zeta are predominantly expressed by progenitor glial cells (Schwann cells, oligodendrocytes, and astroglia) and neurons (76, 77). These data suggest that PTPbeta/zeta plays a role in gliogenesis and in glial-neuron interactions. Perhaps the most interesting characteristic of PTPbeta/zeta is its ability to interact with a variety of neurite-promoting cell adhesion proteins including N-CAM, Nr-CAM, Ng-CAM, tenascin, axonin-1/TAG-1, and contactin/F3, suggesting that PTPbeta/zeta mediates neurite outgrowth and neuronal cell adhesion (21, 73, 78, 79). Initial studies on contactin/F3 and tenascin-C suggest that both bind directly to PTPbeta/zeta, with the highest affinity for the contactin/F3-PTPbeta/zeta interaction (80). The contactin/F3-PTPbeta/zeta interaction induces neurite outgrowth of primary neurons and is mediated by the CAH domain of PTPbeta/zeta (79). On the other hand, the tenascin-C/PTPbeta/zeta interaction is mediated by the FN III domain and plays a role in glial cell adhesion (17). In addition to these CAMs, the heparin binding growth associated molecules, midkine and pleiotrophin (HB-GAM), have been shown to bind PTPbeta/zeta with high affinity (81, 82). Of particular interest is the interaction of PTPbeta/zeta with pleiotrophin which results in lower phosphatase activity, increased beta-catenin phosphorylation, and increased migration of cortical neurons (83). These data suggest that pleiotrophin functions as a physiological modulator of PTPbeta/zeta activity that can directly influence the state of beta-catenin phosphorylation. Through modulation of beta-catenin phosphorylation, PTPbeta/zeta might regulate events such as neuronal migration and/or neurite outgrowth (84). Even though *in vitro* data suggests that PTPbeta/zeta is important for the development of the vertebrate nervous system, PTPbeta/zeta knockout mice show no obvious abnormalities (85). It is possible that PTPgamma, the other type V RPTP, compensates for the lack of PTPbeta/zeta and prevents the development of a marked phenotype. Currently, little is known about PTPgamma, however; some differences with PTPbeta/zeta have been identified. For example, the neurite promoting effects of NGF in PC12D cells can be inhibited by expression of PTPgamma but not PTPbeta/zeta (86). One intriguing aspect of PTPgamma is its chromosomal localization on human chromosome 3p 14.2-p21 a region frequently deleted in certain types of renal and lung carcinomas (87, 88).

In summary, the type I, II and V RPTP act as heterophilic CAMs with one or more ligands. The expression patterns and ligand identities suggest that these RPTPs mediate interactions among cells, and between cells and ECM. In the immune and nervous system, these interactions are important for normal physiologic function and their disruption may be implicated in some diseases.

4. RPTPs AS ORPHAN RECEPTORS

As discussed above, RPTPs can play important roles in cell-cell and cell-ECM adhesion by binding to themselves, to other proteins, or to carbohydrate modifications present in ECM components. The identification of these binding properties has been critical for understanding RPTP function; however, many other binding partners and properties remain to be identified. This is especially true for the type III RPTPs, whose ligands remain elusive. Experimental data suggest that type III RPTPs play important roles in the development of the nervous and immune systems and in the control of epithelial cell growth.

4.1 Type III RPTPs

Among the type III RPTPs present in vertebrates are PTPeta/DEP-1 (density enhanced phosphatase 1) also known as CD148, and PTPRO, also known as GLEPP-1, PTP-BK, PTP-U2 and CRYP-2 (7, 89-94). The structures of these two phosphatases are highly similar with eight extracellular FNIII domains and a single intracellular phosphatase domain. PTPeta/DEP-1 was originally described as a cell-density enhanced phosphatase thought to play a role in mediating "contact-inhibition of cell growth" (90). This phenomenon, critical in maintaining tissue homeostasis, is not observed in transformed cells raising the possibility that PTPeta/DEP-1 may be involved in the inhibition of cellular transformation. In fact, recent studies have shown that PTPeta/DEP-1 expression is downregulated in many thyroid human carcinoma cells and that re-expression of PTPeta/DEP-1 suppresses their malignant phenotype, probably by stabilization of p27/Kip1 (95). In another study, PTPeta/DEP-1 was shown to be upregulated during differentiation and to inhibit growth of breast cancer cells (96). No ligand has yet been identified for PTPeta/DEP-1. However, one recent study suggests that a specific ligand is found on Matrigel, an ECM protein preparation (97). This putative ligand binds to the ECD of PTPeta/DEP-1 and upregulates its intracellular phosphatase activity (97). Identification of this ligand would be of great interest since it would represent the first described positive regulator of RPTP activity. This idea is especially interesting in light of recent data showing that antibody-

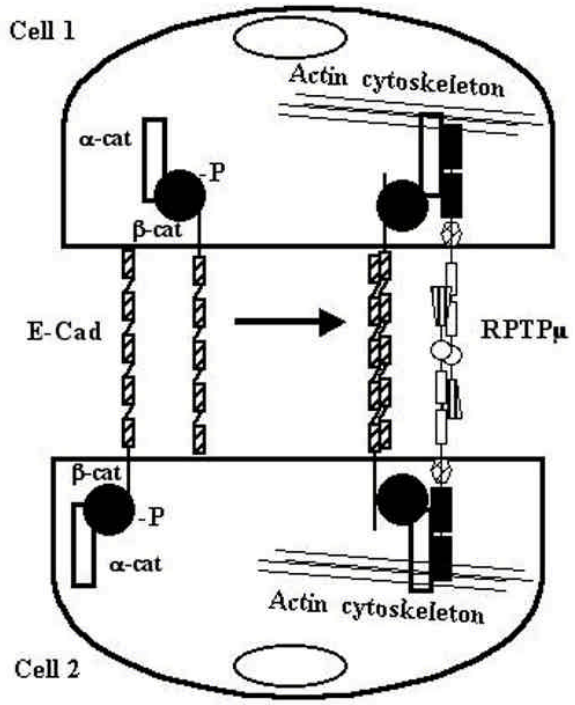

Figure 4. RPTPs regulate the activity of other CAMs by directly modulating their tyrosine phosphorylation levels. The RPTP, PTPmu has been shown to interact homophilically across cell membranes to mediate cell-cell adhesion. It has also been shown to bind to the cadherin/catenin complex intracellularly and modulate its tyrosine phosphorylation. This model shows that when the cadherin/catenin complex is phosphorylated, cell-cell adhesion through the cadherins is downregulated (left). In the presence of PTPmu, the cadherin/catenin complex is dephosphorylated and adhesion is induced both through cadherin and PTPmu homophilic interactions. Under these circumstances, alpha-catenin interacts with the actin cytoskeleton and adhesion junctions are stabilized (right). alpha-cat, alpha-catenin; beta-cat, beta-catenin; E-cad, E-cadherin.

nduced dimerization of PTPeta/DEP-1 appears to increase phosphatase activity (98). PTPeta/DEP-1 is widely expressed on B and T cells, granulocytes, macrophages, dendritic cells, mature thymocytes, fibrocytes, melanocytes, and Schwann cells (99), where it is known as CD148 (100). Its expression is significantly increased in inflamed tissues and after *in vitro* activation of peripheral blood cells (99). Even though the exact function and ligands for CD148 have not been described, it may be involved in leukocyte function as a modulator of TCR/CD3 complex signaling, possibly similar to CD45 (101-103). Recently an important role for CD148 has been identified in vascular organization and development (98). This is the result of observations made in mice lacking catalytically active CD148 and severely hypomorphic for CD148 protein. Mutant mice die at mid-gestation (E11) and show growth retardation and severe angiogenic defects, including highly disorganized structure in the primary vascular plexus of the yolk sac and intraembryonic tissues (98, 104). Of interest is also the observation that PTPeta/DEP-1/CD148 is localized in human chromosome 11p11.2, a region frequently mutated or deleted in human carcinomas (89). The increasing amount of data being collected on this RPTP suggest that it may play a role in controlling adhesive events involved in cellular transformation, immune system cell activation, and angiogenesis. Characterization of ligands for epithelial, endothelial, and immune forms of PTPeta/DEP-1 will without doubt launch this phosphatase as a promising target for drug development, especially in the angiogenesis field.

In contrast to PTPeta/DEP-1, PTPRO expression is highly restricted (91, 92, 94). PTPRO is confined to some regions of the nervous system and to a highly specialized glomerular epithelial cell, the podocyte (92). In the nervous system, PTPRO is highly expressed during embryonic development, especially in the retina and axons of retinal ganglion cells, making this an appropriate location to search for ligands (64). An Fc fusion protein containing the ECD of PTPRO reveals binding sites in most of the E7 neural retina, with the strongest binding observed in the retinal ganglion cell layer, suggesting the presence of a heterophilic binding partner (105). *In vitro*, the ECD of PTPRO is neurite-inhibitory, induces growth cone collapse and is repulsive to growth cones of retinal ganglion cells, suggesting a role in axon guidance (105). *Drosophila* relatives of PTPRO, PTP10D and PTP52F, cooperate and compete with other *Drosophila* RPTPs to control axon growth and guidance (106). In particular, PTP10D has been shown to be involved in the repulsion of axons from the CNS midline. Interestingly, we have observed (PJB and JLB, unpublished) that PTPRO is highly expressed throughout the spinal cord midline but is absent from the floor plate, a region known for allowing commissural axon crossing (107). A scenario in which PTPRO acts as a repulsive adhesion receptor preventing axons from aberrantly crossing the midline can easily be envisioned. However, this possibility remains to be proven. In the kidney, PTPRO is expressed in podocytes, where it is thought to play a role in maintaining foot process structure and/or function by regulating tyrosine phosphorylation of podocyte proteins (92). Support for this hypothesis comes from PTPRO$^{-/-}$ mice which show amoeboid rather than typical octopoid podocyte structures, together with widening of minor foot processes in association with altered distribution of vimentin (108). The human isoform of PTPRO has also been shown to be upregulated during terminal differentiation of myeloid leukemia cells, suggesting that PTPRO may posses a therapeutic role in human malignancies (109, 110). The identification of PTPRO ligands is an area of active investigation. Characterization of such ligands will enhance our knowledge of PTPRO function during axonogenesis, kidney development, and blood cell differentiation.

5. RPTPs AS INDIRECT REGULATORS OF CELL ADHESION

As is the case with ligands, substrate identification for RPTPs has also proven to be a difficult endeavor. This difficulty is probably the result of the transient nature of the interaction between the phosphatases

and their substrates. Lately however, efforts have begun to pay off and some substrates have been identified. Interestingly, some of these substrates are known regulators of cellular adhesion, suggesting that RPTPs not only act as cell adhesion proteins but also regulate cell adhesion through intracellular modification of proteins critical for this process. Here, we will discuss substrates for RPTPs that have known roles as mediators/regulators of cellular adhesion. More general discussions of relevant substrates for RPTPs and their signalling pathways can be found in the recent literature (1, 7, 8).

The best example of a PTPase substrate important in cell adhesion is the cadherin/catenin complex (Figure 4) (48). The classical cadherins are a well-studied family of homophilic, calcium-dependent adhesion molecules whose signaling is mediated by the catenins (35, 111, 112). Four distinct catenins have been identified. Three of them, beta-catenin, gamma-catenin, and $p120^{ctn}$, bind to the intracellular domains of cadherins, while alpha-catenin links the other catenins to the actin cytoskeleton (48). Cadherin-catenin interactions are essential for cadherin mediated cell-cell adhesion as is linkage to the actin cytoskeleton. Tyrosine phosphorylation of both catenins and cadherins is inversely related to cadherin-mediated adhesion (113). Therefore, RPTPs can potentially induce cadherin mediated cell-cell adhesion by dephosphorylating both cadherins and catenins. This idea is supported by the presence of a cadherin-like, catenin-binding domain in the intracellular juxtamembrane region of type IIb RPTPs and by the observation that these molecules co-localize in adherens junctions (43, 114). Type IIa RPTPs (LAR), type IIb RPTPs (PTPmu, PTPkappa and PTPlambda?and type V RPTPs (PTPbeta/zeta? have been shown to associate with the cadherin/catenin complex (44, 83, 115-117). Even though the data are complex and somewhat controversial, it is clear that physiologically significant interactions occur between these RPTPs and the cadherin/catenin complex (49, 118). Current data indicate that PTPmu interacts with and dephosphorylates $p120^{ctn}$ and can also interact directly with the E, N and R cadherins (43, 44, 115). In contrast, PTPkappa and PTPlambda, also type IIb RPTPs, interact with beta and gamma-catenin but do not appear to interact with cadherins (118). The same is the case for LAR, a type IIa RPTP (117). PTPbeta/zeta?interacts with and modulates beta-catenin phosphorylation in a manner controlled by pleiotrophin binding (83). Interactions between RPTPs and cadherin/catenin complexes are of great interest because disruptions in the function of the cadherin/catenin complex have been identified in many human cancers (119, 120). RPTPs now represent one more level at which this complex might be disrupted. The involvement of RPTPs in regulating cadherin complexes is another link between these proteins and cellular transformation and metastasis (47, 121).

Also important for the study of cell adhesion is the interaction of LAR with $p130^{cas}$. $P130^{cas}$ is an adaptor protein localized to focal adhesions that can be directly phosphorylated by Src. $P130^{cas}$ also binds other adhesion regulatory proteins including Lyn, Crk and FAK (122-124). The ability of $p130^{cas}$ to interact with many proteins at the focal adhesion complex gives it a central role in the regulation of cell migration and adhesion. LAR dephosphorylates $p130^{cas}$ reducing its stability and inducing cell death (125). Another protein present in focal adhesions is paxillin. $P130^{cas}$ associates with paxillin and allows the recruitment of effector molecules such as Crk which transduce external signals into changes in cell motility (126). Interestingly, PTPphi, which is an intracellular splice variant of PTPRO, has been found to regulate tyrosine phosphorylation of paxillin in macrophages (127). In addition, paxillin interacts with GTPase-activating proteins of the ARF family of small GTPases, including the GRK interactor 1 (GIT1). The functional results of this interaction are not completely clear, but GIT1 appears to function in a paxillin-containing complex that regulates cell motility and cellular adhesion (128). Using a substrate-trapping mutant together with a yeast two-hybrid system, GIT1 has been isolated as a substrate for the type V RPTP, PTPbeta/zeta. The association between GIT1 and PTPbeta/zeta seems to depend on the tyrosine phosphorylation of GIT1 (129). These data present a scenario in which PTPbeta/zeta may indirectly modulate cell adhesion by regulating the free pool of GIT1 available to interact with paxillin and other elements of adhesion complexes and the membrane skeleton. These results together suggest that RPTPs are involved in controlling the activity of focal adhesions, and thus cellular adhesion and motility, by regulating the tyrosine phosphorylation levels of its member proteins.

6. PERSPECTIVE

RPTPs represent an exciting group of proteins possessing the ability to directly translate extracellular adhesion events into cytoplasmic signals. Even though the functional characterization of RPTPs is still in its infancy, great progress is being made. The identification of physiologically relevant ligands and substrates is perhaps the most important short-term goal in this field. Knowledge of new ligands that upon binding to RPTPs induce changes in phosphatase activity is critical for our understanding of RPTP function. These ligands not only will allow us to perform detailed structural and biochemical analyses to understand activity regulation, but will also provide spatial and temporal cues to study the physiological roles of RPTPs. Furthermore, the identification of substrates will allow us to understand how these complex molecules signal to the nucleus. Most importantly, ligands and substrates will provide invaluable information for the design of synthetic targeting drugs that can modulate (mimic or inhibit) the activities of RPTPs and help reverse pathologic events resulting from disturbances in the expression or activity of these enzymes. Synthetic drugs, in the form of small molecules, are currently being designed against the active site of PTP1B, a cytoplasmic PTP that has been identified as a promising target for anti-diabetes and obesity drugs (130).

7. ACKNOWLEDGEMENTS

Work on tyrosine phosphatases in the authors' laboratory is supported by the NIH and the NSF. PJB is a

Lois Pope LIFE Postdoctoral Fellow. The authors benefited from conversations with Drs. Thomas O. Daniel and Masaharu Noda.

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Abbreviations:
CAH: Carbonic anhydrase
CAM: Cell adhesion molecule
CNS: Central nervous system
ECD: Extracellular domain
ECM: Extracellular matrix
FNIII: Fibronectin type III
HSPG: Heparan sulfate proteoglycan
Ig: Immunoglobulin
MAM: Meprin, A5, PTPμ
PTK: Protein tyrosine kinase
PTP: Protein tyrosine phosphatase
RPTP: Receptor protein tyrosine phosphatase
TCR: T-cell receptor

Key Words: Cell Adhesion Molecules, Axon Growth, Cancer Biology, Cadherins, Vascular Biology, Review

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