Published in final edited form as:

Cell Commun Adhes. 2010 April ; 17(2): 34–47. doi:10.3109/15419061.2010.487957.

Characterization of the adhesive properties of the type IIb subfamily of RPTPs

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Abstract

Receptor protein tyrosine phosphatases (RPTPs) have cell adhesion molecule-like extracellular domains coupled to cytoplasmic tyrosine phosphatase domains. PTPmu is the prototypical member of the type IIb subfamily of RPTPs, which includes PTP rho, PTP kappa, and PCP-2. We performed the first comprehensive analysis of the subfamily in one system, examining adhesion and antibody recognition. We evaluated if antibodies that we developed to detect PTPmu also recognized other subfamily members. Notably, each antibody recognizes distinct subsets of type IIb RPTPs. PTPmu, PTP rho and PTP kappa have all been shown to mediate cell-cell aggregation, and prior work with PCP-2 indicated that it can mediate bead aggregation *in vitro*. This study reveals that PCP-2 is unique among the type IIb RPTPs in that it does not mediate cell-cell aggregation via homophilic binding. We conclude from these experiments that PCP-2 is likely to have a distinct biological function other than cell-cell aggregation.

Keywords

Receptor protein tyrosine phosphatase; PCP-2; cell-cell aggregation; tyrosine phosphatase; homophilic binding; cell adhesion

1. INTRODUCTION

Receptor protein tyrosine phosphatases (RPTPs) comprise a family of proteins with extracellular cell adhesion molecule (CAM) domains coupled to cytoplasmic tyrosine phosphatase domains, and thus can influence many biological processes, including cancer progression (Ostman et al. 2006). The type IIb PTPμ-like subfamily of RPTPs includes PTPμ, PTPρ, PTPκ, and PCP-2 (PTPλ) (Brady-Kalnay 2001). The extracellular segment of the PTPμ-like RPTPs contains a Mephrin-A5 (neuropilin)-μ (MAM) domain, an immunoglobulin (Ig) domain and four fibronectin type III (FNIII) repeats (Brady-Kalnay 2001; Ostman et al. 2006). Their intracellular segments contain a juxtamembrane domain with sequence similarity to the cytoplasmic domain of classical cadherins, as well as two tandem tyrosine phosphatase domains. Globally, type IIb RPTPs share approximately 55% sequence identity, whereas their cytoplasmic domains share approximately 68% sequence identity.

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PTP μ subfamily members bind homophilically (i.e. the “ligand” for PTP μ is an identical PTP μ molecule on an adjacent cell) (Aricescu et al. 2008; Brady-Kalnay et al. 1993; Cheng et al. 1997; Gebbink et al. 1993; Sap et al. 1994; Yu et al. 2008; Zondag et al. 1995). The Ig domain of PTP μ is responsible for promoting homophilic interactions (Brady-Kalnay and Tonks 1994), and proper cell surface localization (Del Vecchio and Tonks 2005). However, various laboratories have demonstrated that both the MAM and Ig domains as well as the first two FNIII repeats play a role in efficient cell-cell adhesion of PTP μ (Aricescu et al. 2006; Aricescu et al. 2007; Brady-Kalnay and Tonks 1994; Zondag et al. 1995). PTP ρ and PTP κ also mediate cell-cell adhesion via homophilic binding (Sap et al. 1994; Yu et al. 2008). To date, extracellular fragments of PCP-2 have only been demonstrated to mediate aggregation of beads *in vitro* (Cheng et al. 1997). Despite the amino acid sequence similarity between type IIb RPTPs, no heterophilic interactions between the subfamily members have been observed. For example, PTP μ does not interact heterophilically with either PTP κ (Zondag et al. 1995) or PTP ρ (Yu et al. 2008).

PCP-2 was originally identified in a human pancreatic adenocarcinoma cDNA library, and was shown to localize to adherens junctions (Wang et al. 1996). PCP-2 is predicted to be a tumor suppressor gene based on its reduced expression in melanoma cell lines and tissue biopsies (McArdle et al. 2001). Like other type IIb RPTPs (Lilien 2005; Sallee et al. 2006), PCP-2 also regulates the cadherin-catenin complex. PCP-2 interacts with and dephosphorylates β -catenin *in vivo*, thereby promoting E-cadherin-mediated adhesion (Yan et al. 2002; Yan et al. 2006). While structural studies suggest there is little difference between type IIb RPTPs, whether PCP-2 is capable of mediating cell-cell adhesion and thus capable of functioning like other type IIb RPTPs remains to be tested.

In this study, we tested the specificity of PTP μ antibodies that we generated and are now commercially available. Our findings demonstrate that each PTP μ antibody recognizes distinct members of the type IIb subfamily of RPTPs. Based on recombinant PTP μ fragment binding experiments and sequence comparison, we were able to predict the epitopes on the type IIb RPTPs that the antibodies bind to. In addition, we characterize the ability of PCP-2 to mediate cellular aggregation. Our results demonstrate that PCP-2 is unique among the type IIb RPTPs in its inability to mediate cell-cell aggregation. Domain swapping experiments demonstrate that either the MAM or Ig domain of PCP-2 is capable of weakly substituting for the homologous domain when inserted into otherwise wild-type PTP ρ . We compared the type IIb subfamily members using homology modeling based upon the PTP μ crystal structure. This modeling illustrates the unique surface charges of the adhesive interfaces of each type IIb subfamily member, which may account for the fact that no heterophilic binding is observed in this subfamily. In summary, the experiments described in this manuscript emphasize that despite sharing enough sequence similarity to be recognized by some of the same antibodies, the type IIb RPTPs function in distinct manners biologically. Our data suggest that PCP-2 has a function other than mediating cell-cell aggregation.

2. METHODS

2.1 Antibodies

The following monoclonal antibodies, generated using the intracellular domain of PTP μ as the antigen, were used in this study: SK6, SK7, SK10, SK15, and SK18 (Brady-Kalnay et al. 1993; Brady-Kalnay and Tonks 1994). A monoclonal antibody to the C-terminal portion of PTP μ (termed C-terminal PTP μ antibody) was also used (Gebbink et al. 1991). The C-terminal antibody was generated using a synthetic peptide of amino acids 1436–1452 corresponding to the predicted C-terminus of PTP μ (Gebbink et al. 1991).

2.2 Baculovirus generation

Baculoviruses were generated by transfecting cells with the BaculoGold™ Linearized Baculovirus DNA (BD Biosciences, San Diego, CA) according to the manufacturer's protocol. Baculoviruses for PTP μ (Brady-Kalnay et al. 1993) and PTP ρ (Yu et al. 2008) were previously described. PTPRK-pBluescript plasmid (kind gift from Dr. Jan Sap) was digested with EcoRI and HpaI and ligated into the pVL1393 vector digested with EcoRI and SmaI. Full length PCP-2 was removed from the pMT2-PCP-2 construct using NotI and XbaI and ligated into the pVL1392 vector digested with NotI and XbaI. The GFP baculovirus pVL1393-GFP was obtained from BD Biosciences. The RFP baculovirus was generated by cloning the pmRFP1 coding sequence (Campbell et al. 2002) into pVL-1393 using the restriction sites BamHI and EcoRI.

The PTP ρ -PCP-2 MAM and Ig chimeras were generated by fusion PCR as previously described (Higuchi et al. 1988). Briefly, taking the PTP ρ -PCP-2 MAM chimera as an example, the MAM domain of PCP-2 was amplified from a PCP-2 plasmid. In order to perform the cloning, two fragments were amplified from pVL1392-PTP ρ at the same time. One fragment encodes the PTP ρ signal sequence starting from the EcoRV site, which is in the vector, just 5' to the MAM domain of PTP ρ . An overhang of eighteen nucleotides that corresponds to the first eighteen nucleotides of the MAM domain of PCP-2 was introduced to the 3' end of the fragment by the reverse primer. The other primer starts at the sequence at the 3' end of the MAM domain of PTP ρ to the BglII site, which is the closest restriction site available in the DNA sequence of PTP ρ . An overhang of eighteen nucleotides that matches the last eighteen nucleotides of the MAM domain of PCP-2 was introduced to the 5' end of the fragment by the forward primer. The PCR products were mixed and used as templates for another round of PCR to generate PTP ρ -PCP-2 MAM chimeric DNA. The fused PCR products were cloned into pVL1392-PTP ρ using EcoRV and BglII. No additional amino acids were introduced as confirmed by DNA sequencing.

The PCP-2 MAM domain: forward primer 5'-cccgccgccccgggctcagactccggcagctggctgc-3'; reverse primer 5'-tcgcagaaaatgaggtgcctttgctgcagggtagct-3'. Two flanking sequence of PTP ρ : forward primer-1 5'-ttgctgatatcatggagataaataaaa-3', reverse primer-15'-gcagccagctgccggagctgagccccggcgccggg-3' or forward primer-2 5'-agctaccctgcgcaaaggcacctcattttctgcga-3' and reverse primer-2 5'-cctttgaggagaagatctacatcc-3'. Primers used to fuse these three fragments together: forward primer 5'-ttgctgatatcatggagataaataaaa-3' and reverse primer 5'-cctttgaggagaagatctacatcc-3'.

PTP ρ -PCP-2 Ig Chimera was generated using the same strategy as used for MAM swapping mutant except that the primers are as follows: The PCP-2 Ig domain: forward primer 5'-gcacctcattttctgcgactggcgacgtggagtc-3'; reverse primer 5'-aatggcgctgggaggtccttgacgatgagctccgc-3'. Two flanking sequences of PTP ρ : forward primer-1 5'-ttgctgatatcatggagataaataaaa-3'; reverse primer-1 5'-gacctccagctgcccgagctgcagaaaatgaggtgc-3' and forward primer-2 5'-gcggagctcatcgtcaaggagcctccacgcccatt-3' and reverse primer-2 5'-cctttgaggagaagatctacatcc-3'. Primers used to fuse three fragments together: forward primer: 5'-ttgctgatatcatggagataaataaaa-3'; reverse primer 5'-cctttgaggagaagatctacatcc-3'.

PTP ρ -PCP-2 extracellular chimera: Extracellular domain of PCP-2 was amplified from a PCP-2 plasmid using forward primer 5'-tgaccagaattcatggcccggtgccaggcg-3' and reverse primer 5'-tgaccagaattctccttgaggcagcttcc-3' starting from the start codon ATG to the sequence 5' of the EcoRI site in the PTP ρ gene, which is very close to the transmembrane domain. The fragment was cloned into pVL1392-PTP ρ using EcoRI, which was introduced into the primers.

2.3 Sf9 cell culture

Sf9 insect cells (BD Biosciences, San Jose, CA) were maintained at 27°C in Graces Insect Medium (BD Biosciences). Medium was supplemented with 10% FBS (Hyclone, Logan, UT) and Gentamicin (MP Biomedicals, Solon, OH). Cells were plated in T25 flasks and one ml of the viruses described above were added to Sf9 cell medium to induce the expression of proteins of interest. Cells were harvested 30 hours post-infection for further analysis. For heterophilic cell-cell aggregation assays, co-infection with 1ml of RFP or GFP virus and 1 ml of PTP virus was performed by simultaneous addition to the Sf9 cell culture. The aggregation assays were performed 48 hours post-infection for maximal fluorescent protein expression.

2.4 Immunoblotting

Sf9 cells were lysed in a standard lysis buffer (20 mM Tris-HCl, pH 7.5, 1% Triton X-100, 150 mM sodium chloride, 2 mM Benzamidine, 2.5µg/ml Aprotinin, 2.5µg/ml Leupeptin, and 0.5µg/ml Pepstatin). Samples were solubilized in an equal volume of 2X SDS loading buffer and separated by SDS-PAGE. Samples were transferred to nitrocellulose membrane (Whatman, Piscataway, NJ) and immunoblotted with anti-PTPµ antibodies as described (Brady-Kalnay et al. 1993).

2.5 Immunocytochemistry

Sf9 cells were fixed with 2% paraformaldehyde diluted in 1X PBS for 30 minutes at room temperature. Cells were permeabilized and blocked with 0.5% saponin containing 10% goat serum diluted in 1X PBS for 45 minutes. Cells were incubated with mouse monoclonal anti-PTPµ (SK15) antibody for one hour then washed followed by addition of Texas Red-conjugated goat anti-mouse IgG secondary antibody (Jackson ImmunoResearch Laboratories, Inc. West Grove, PA) for one hour, washed and imaged. Images were captured with a Nikon TE-200 inverted fluorescence microscope.

2.6 Cell surface expression of RTPs

Cell surface expression of PTPµ, PTPρ, PTPκ, and PCP-2 proteins in baculovirus infected Sf9 cells was determined using a modified biotin-avidin reaction with the Cell Surface Protein Isolation Kit (Pierce, Rockford, IL), as previously described (Yu et al. 2008). Briefly, Sf9 cell media was removed and the flasks were incubated with rocking for 30 minutes at 4°C in 5ml of 0.25mg/ml Sulfo-NHS-SS-Biotin in 1X PBS. The cells were mechanically removed from the flask, centrifuged, washed, and lysed according to the manufacturer's protocols. To isolate cell surface proteins, the cell lysate was added to a NeutrAvidin Gel column and incubated for 60 min at room temperature (RT) with rocking prior to washing and eluting proteins with 200µl of sample buffer containing a final concentration of 55mM DTT for 60min at RT. The flow through was run on 6% SDS-PAGE gels and transferred to nitrocellulose. Total cell surface protein levels were normalized by immunoblotting cell lysates with an anti-actin antibody (JLA20). The normalized cell surface protein was run on 6% SDS-PAGE gels, transferred to nitrocellulose and immunoblotted with antibodies to PTPµ (SK7, SK15 or SK18) or actin (JLA20) followed by secondary antibody conjugated to HRP. The HRP signal was detected using a Fluor-S MAX MultiImager (Bio-Rad Laboratories Inc., Hercules, CA).

2.7 Expression of Fusion Proteins in E. coli

Plasmids containing the intracellular GST-PTPµ fusion proteins, GST-iPTPµ:765–1452, GST-iPTPµ:765–1178 and GST-PTPµ:765–958 GST-iPTPµ:816–1452 (iPTPµ-Δ765–815) were previously described (Phillips-Mason et al. 2006). These fusion proteins were

expressed in bacteria under the control of the *lac* operon and isolated on glutathione Sepharose 4B (GE Healthcare, Piscataway, NJ).

The pTrcHis plasmid was used to generate T7-6(His)-tagged PTP μ fusion proteins. Generation of His-iPTP μ : 774–1161, His-iPTP μ : 1213–1452, and His-iPTP μ : 915–1161 have been described (Phillips-Mason et al. 2008). Using PCR, we generated a plasmid encoding most of the juxtamembrane domain (His-iPTP μ : 774–924) that expresses a T7/His-tagged fusion protein containing amino acids 774–924 of PTP μ . The T7/His-tagged fusion proteins were expressed in *E. coli* under the control of the *lac* operon. These proteins were purified using the PrepEase His-Tagged Protein Purification Kit (USB Corporation, Cleveland, OH).

2.8 Aggregation Assays

Sf9 cells were harvested by trituration. The cell suspensions were added to glass scintillation vials and incubated at 27°C at 90 rpm in a gyratory shaker for 30 minutes. For a given condition, the cell suspension was transferred to a 100mm Petri dish either prior to (0 time point) or 30 minutes after aggregation. Images were captured with a Nikon TE-200 inverted fluorescent microscope (Tokyo, Japan). To quantify cell aggregates, pictures of four randomly selected fields were taken per dish. The area of each object (single cells and aggregates) was determined by the Metamorph software (Molecular Devices, Downingtown, PA) using an auto-threshold setting for light objects and appropriate size filters that allowed the counting of cells and aggregates but not debris. The four replicates were combined to yield average readings per condition. The percent aggregation was then calculated as the average aggregate area at 30 minutes minus the average aggregate area at zero minutes divided by the average aggregate area at 30 minutes $[(N_{30}-N_0)/N_{30}]$. A minimum of three independent experiments was performed per condition. Statistical significance was determined in Microsoft Excel using Student's T-test. Error bars indicate standard error.

2.9 Surface Electrostatic potential calculations of type IIb RPTPs

Homology models were generated by submitting the human protein sequence for the PTP of interest to the phyre protein homology server (<http://www.sbg.bio.ic.ac.uk/phyre>). Resultant models were inspected and corrected in Coot (<http://www.biop.ox.ac.uk/coot/>) with structural idealization in REFMAC (<http://www.ysbl.york.ac.uk/~garib/refmac/>). APBS (<http://www.poissonboltzmann.org/apbs/>) was utilized in concert with PyMOL (Schrödinger, New York, NY) to calculate and visualize electrostatic surface potentials.

3. RESULTS

3.1 Cross-reactivity of PTP μ intracellular antibodies

Previously, a series of antibodies termed the SK antibodies were generated using the entire intracellular domain of PTP μ as an antigen (Brady-Kalnay et al. 1993). The cytoplasmic domains of the type IIb RPTPs share approximately 55% sequence identity at the amino acid level (Brady-Kalnay 1998). Given this high degree of similarity, it is likely that these antibodies cross-react with other type IIb subfamily members and may serve as useful reagents for studies evaluating these RPTPs as well. To test this, type IIb RPTPs were expressed in Sf9 insect cells by infecting them with baculovirus encoding the different RPTPs. Sf9 cell lysates were then probed with an antibody generated by using a peptide from the C-terminus of PTP μ , termed the C-terminal antibody (Gebbinck et al. 1991) and the SK series monoclonal antibodies: SK6, SK7, SK10, SK15, SK18 (Brady-Kalnay et al. 1993) (Figures 1A and 1B). PTP μ intracellular antibodies recognize proteins at the expected molecular weight of type IIb RPTPs, approximately 200kD. All of these antibodies recognize PTP μ . Of the six PTP μ antibodies, three, SK6, SK10, and the PTP μ C-terminal

antibody, exclusively bound to PTP μ (Figure 1A). In addition to recognizing PTP μ , SK18 recognizes PTP ρ , SK7 recognizes PTP κ and PCP-2, and SK15 recognizes PTP ρ and PCP-2 (Figures 1A and C). All of these proteins, as expected, were expressed on the surface of Sf9 cells (Figure 1B).

3.2 Identification of the binding regions of the SK series of PTP μ intracellular antibodies

To identify the binding regions of the PTP μ intracellular antibodies used above, the antibodies were tested for their ability to recognize recombinant PTP μ cytoplasmic domain fusion proteins in immunoblot experiments. All antibodies examined recognized the complete cytoplasmic domain fusion protein GST-iPTP μ : 765–1452, encoding the juxtamembrane domain and two tyrosine phosphatase domains, containing amino acids (AA) 765–1452 (Figures 2A and B). SK6, SK7, and SK10 all recognized the same PTP μ fusion proteins and failed to recognize the fusion proteins lacking residues within the juxtamembrane domain, His-PTP μ : 915–1161 and His-PTP μ : 1213–1452 (Figure 2). SK15 did not recognize fusion proteins lacking the first phosphatase domain, including GST-iPTP μ : 765–958, His-iPTP μ : 1213–1452 or His-iPTP μ : 774–924 (Figure 2). SK18 also did not recognize fusion proteins lacking the first phosphatase domain, including His-iPTP μ : 1213–1452 or the His-iPTP μ : 774–924 (Figure 2). Unlike SK15, however, SK18 did recognize the fusion protein GST-iPTP μ : 765–958 (Figure 2) indicating that the binding site resides between residues 925 and 958. As a control, the PTP μ C-terminal antibody was also evaluated in our studies. The C-terminal PTP μ antibody was generated using a peptide of residues 1436–1452. As is expected, this antibody did not recognize proteins lacking the membrane distal phosphatase domain, which contains residues 1436–1452 (Figure 2). These results indicate that the epitopes recognized by the different PTP μ antibodies are localized to the following sequences: SK6, SK7 and SK10: 816–914; SK15: 958–1161; SK18: 925–958; the C-terminal PTP μ antibody: 1436–1452.

3.3 Identification of SK antibody series binding sites by amino acid sequence comparison of type IIb RPTP subfamily members

The specificity of PTP μ intracellular antibodies for type IIb RPTPs (Figure 1) and the ability of these antibodies to bind to various PTP μ fusion proteins (Figure 2B) were cross referenced with the RPTP type IIb cytoplasmic domain sequences (Figure 3). Using this information, we made predictions as to where the PTP μ intracellular antibodies likely bind. For example, to determine the epitopes for the PTP μ specific antibodies SK6 and SK10, we identified regions of amino acid differences between PTP μ and other type IIb subfamily members within the predicted epitope domains determined using the fusion protein experiments. On the other hand, for antibodies which cross-reacted with other subfamily members, we looked for regions of greatest similarity between the different members to identify the most likely region of epitope binding. SK6 and SK10 binding was eliminated when residues 816–914 were truncated from intracellular PTP μ fusion proteins (Figure 2). Based on the lack of cross-reactivity with other type IIb RPTPs (Figure 1A), SK6 and SK10 are predicted to bind to residues 816–867, a region with little homology to other type IIb RPTPs (Figure 3A, black box). SK7 also did not recognize the recombinant PTP μ proteins lacking residues 816–914 (Figure 2). In cross-reactivity studies, SK7 recognized PTP μ , PTP κ , and PCP-2 (Figure 1), suggesting that the epitope is a region of sequence similarity between these three RPTPs, which could either be between residues 850–855 or residues 868–877 (Figure 3B, black box). SK15 did not bind to intracellular PTP μ truncations lacking residues 958–1161 within the first phosphatase domain (Figure 2). SK15 also recognizes PTP μ , PTP ρ , and PCP-2 (Figure 1). The region between residues 958–1161 has a high degree of similarity between all subfamily members. Therefore, we could not further narrow down the binding site of SK15 within this region (Figure 3C). SK18 did not recognize the fusion proteins His-iPTP μ : 1213–1452 or the His-iPTP μ : 774–924 which lack

amino acids within the first phosphatase domain but did recognize GST-iPTP μ : 765–958 (Figure 2). PTP μ and PTP ρ but not PCP-2 were recognized by SK18 (Figure 1). The SK18 binding region is predicted to be an area of least sequence similarity with PCP-2 somewhere between residues 924–958, most likely 941–947 (Figure 3D). The PTP μ C-terminal antibody did not bind any PTP μ intracellular truncations that lacked the membrane distal phosphatase domain (Figure 2), in agreement with the fact that this antibody was generated using a synthetic peptide of residues 1436–1452 corresponding to the predicted C-terminus of PTP μ (Figure 3E) (Gebbink et al. 1991). Our analysis did not allow us to narrow down the epitope further than these 16 residues. Our studies indicate that SK6, SK10 and the C-terminal antibody are highly specific to PTP μ . In addition, our antibody analysis indicates that small variations between the subfamily members in the juxtamembrane domain and/or membrane distal phosphatase domain are sufficient to prevent antibodies that bind to epitopes within these domains from recognizing all subfamily members.

3.4 PCP-2 does not mediate cell-cell aggregation *in vivo*

Our antibody studies demonstrate a high degree of amino acid sequence similarity and cross-reactivity of the PTP μ -subfamily members. Given this high degree of similarity we wanted to examine whether all of the subfamily members are able to mediate cell-cell adhesion. Previous studies demonstrated that PTP μ , PTP ρ and PTP κ all induce cell-cell aggregation of non-adhesive Sf9 insect cells (Brady-Kalnay et al. 1993; Cheng et al. 1997; Gebbink et al. 1993; Sap et al. 1994a; Yu et al. 2008; Zondag et al. 1995). To date, PCP-2 had not been tested for its ability to mediate aggregation of cells. To this end, Sf9 cells were infected with baculovirus to induce expression of PTP μ , PTP ρ , PTP κ , or PCP-2. RPTP expressing Sf9 cells were then used in aggregation assays. We confirmed that PTP μ , PTP ρ and PTP κ mediate Sf9 cell-cell aggregation (Figure 4). Unlike other type IIb RPTPs, however, PCP-2 did not induce cell-cell aggregation when expressed in Sf9 cells (Figure 4).

3.5 PCP-2 does not interact heterophilically to mediate cell-cell aggregation

CAMs can bind heterophilically to mediate adhesion (Duguay et al. 2003; Niessen and Gumbiner 2002). To test whether PCP-2 may bind heterophilically to other type IIb RPTPs to mediate cell-cell aggregation, one set of Sf9 cells was co-infected with viruses expressing full-length PCP-2 and green fluorescent protein (GFP) while another set of Sf9 cells was co-infected with viruses expressing either full-length PTP μ , PTP ρ or PTP κ and red fluorescent protein (RFP). The PCP-2 expressing cells were mixed with an equal number of cells that express PTP μ , PTP ρ or PTP κ and tested in aggregation assays. As shown in the overlay image of Figure 5, the PCP-2 labeled cells did not bind heterophilically or aggregate with the PTP μ , PTP ρ or PTP κ expressing cells.

3.6 PTP κ expressing cells sort away from cells expressing other type IIb RPTPs

Cells expressing PCP-2 do not aggregate with cells that express other type IIb RPTPs. We wanted to evaluate whether the same was true for another type IIb RPTP, PTP κ . To evaluate whether cells that express PTP κ sort away from cells that express other type IIb RPTPs, aggregation assays were conducted by mixing equal numbers of cells expressing either PTP κ and PTP μ , or PTP κ and PTP ρ . Using the overlay image of the co-infected fluorescent protein expression to distinguish the two populations of cells as described above, we found that Sf9 cells expressing PTP κ sort away from Sf9 cells that express either PTP μ (Figure 6), in agreement with previously published findings using lipophilic dyes (Zondag et al. 1995), or PTP ρ to exclusively form aggregates of cells that express only one type IIb RPTP (Figure 6). These results are similar to our recent study demonstrating that PTP ρ and PTP μ sort into independent aggregates (Yu et al. 2008). To date, no heterophilic binding of type IIb RPTPs capable of mediating cell-cell aggregation has been observed. A control experiment with Sf9 cells expressing only RFP or GFP demonstrated that these two fluorescent proteins do not

induce Sf9 cell aggregation. To confirm the co-expression of both the fluorescent protein and the phosphatase, we performed immunocytochemistry on Sf9 cells co-infected with the two proteins (Figure 6). The overlay images of the PCP-2/GFP or PTP μ /GFP co-infected cells demonstrate that the Sf9 cells express both the phosphatase and GFP.

3.7 Swapping the MAM or Ig domains of PCP-2 into PTPp creates a unique protein capable of mediating weak cell-cell aggregation

In order to identify whether the MAM or Ig domains of PCP-2 are functionally capable of mediating cell-cell adhesion, we conducted domain swapping experiments between PCP-2 and PTPp. We made domain swapping mutants between PTPp and PCP-2 based upon our recent studies on the ability of PTPp to mediate cell adhesion (Yu et al. 2008; Zhang et al. 2010). We swapped the MAM, Ig, or entire extracellular domain of PCP-2 into an otherwise wild-type PTPp expression vector and expressed the chimeric proteins in Sf9 cells. All constructs were efficiently expressed and localized to the cell surface (Figure 7). As a control for the cell surface labeling experiments, we verified that actin was expressed in the cell lysates but not in the cell surface labeling samples (Figure 7B). We then analyzed whether the chimeras were capable of mediating cell-cell aggregation. Both the chimeric PTPp/PCP-2 MAM and the PTPp/PCP-2 Ig proteins were able to mediate cell-cell aggregation, although at a much reduced level than wild-type PTPp (Figure 8). The PTPp/PCP-2 MAM chimera mediated 35% cell-cell aggregation and the PTPp/PCP-2 Ig chimera mediated 21% cell-cell aggregation, compared to 69% cell-cell aggregation mediated by wild-type PTPp (Figure 8). The PTPp/PCP-2-Extra chimera did not mediate aggregation (Figure 8) indicating the defect lies in the PCP-2 extracellular domain.

We also conducted cell-sorting experiments with the chimeric PTPp/PCP-2 proteins and with wild-type PTPp. PTPp sorted away from all chimeric proteins, demonstrating that the specificity for homophilic interaction is encoded within the MAM/Ig unit. The chimeras are unique homophilic binding proteins that are not able to interact with wild-type PTPp (Figure 9). Similar results were observed in chimeric studies of PTP μ and PTP κ using lipophilic dyes (Zondag et al. 1995). The data suggest that together the MAM/Ig unit generates a unique binding site within the chimeric protein. The table shown in Figure 9 summarizes all heterophilic binding aggregation assays performed to date and demonstrates that no heterophilic binding has ever been observed in aggregation assays for the type IIb RPTPs.

3.8 Electrostatic calculations of type IIb subfamily homology models depict distinctions in surface charges in the MAM and Ig domains

Based upon crystal contacts observed in the crystal structure of PTP μ , Aricescu and colleagues concluded that the adhesive interface of the PTP μ dimer was between the MAM and Ig domain of one PTP μ molecule and the first and second FNIII domains of another (Aricescu et al. 2007). We therefore wanted to evaluate the surface charge of exposed residues in PCP-2 and compare it to that of other type IIb RPTPs. Using the Phyre protein homology server to generate homology models, we were able to compare the surface charges of the different type IIb RPTPs (Figure 10). These results demonstrate distinct differences in electrostatic surface potential, especially on the backside of the MAM-Ig domain surface, which was predicted to be important for the adhesive interface of type IIb subfamily RPTPs (Aricescu et al. 2007) (Figure 10).

4. DISCUSSION

The exact domain necessary for mediating homophilic binding *in vitro* and cell-cell aggregation via homophilic binding of the prototypical type IIb RPTP, PTP μ , has been a topic of study for 15 years. Homophilic binding of PTP μ -coated covaspheres requires only

the Ig domain of PTP μ (Brady-Kalnay and Tonks 1994). Homophilic cell-cell aggregation, on the other hand, requires the MAM domain, Ig domain and the first two FNIII repeats of PTP μ based upon a series of studies by multiple labs (Aricescu et al. 2006; Aricescu et al. 2007; Brady-Kalnay and Tonks 1994; Zondag et al. 1995). Furthermore, the specificity of type IIb RPTP homophilic cell-cell aggregation is determined in part by the MAM domain (Zondag et al. 1995). There is evidence that suggests that MAM binding is required for *cis* interactions between PTP μ molecules and not for *trans* interactions necessary to mediate cell-cell adhesion. For example, Cismasiu and colleagues demonstrated that when a mutant form of the MAM domain is expressed in cells, binding of PTP μ to the MAM domain is abolished, but cell-cell aggregation is not (Cismasiu et al. 2004).

The sequence identity between the PTP μ -subfamily members is approximately 55% in the extracellular domain. However, the data presented here shows a large structural similarity between type IIb RPTP subfamily members, and indicates that PCP-2 likely has distinct surface charges in the vicinity previously identified as being important to mediating trans-dimer formation (Aricescu et al. 2007). Nevertheless, we were surprised to find that PCP-2 does not mediate cell-cell aggregation. To identify the domains of PCP-2 responsible for deficient cell-cell adhesion, we conducted domain swap experiments between PCP-2 and PTP ρ . When the MAM or Ig domains of PCP-2 are swapped into otherwise wild-type PTP ρ they are able to mediate weak cell-cell aggregation, albeit to a lesser extent than wild-type PTP ρ . This suggests that the MAM and Ig domains of PCP-2 can function within the context of the other functional extracellular domains of PTP ρ . This study also suggests that the MAM and Ig domains each confer specificity to homophilic binding due to the fact that the PTP ρ /PCP-2 MAM or PTP ρ /PCP-2 Ig chimeras sorted away from wild-type PTP ρ (Figure 9).

Analysis of the crystal packing of PTP μ dimers suggests that 17 residues are likely important in mediating the adhesive interface between two PTP μ molecules (as shown in Figures 2C and 2D in Aricescu et al. 2007). Mutation of a selection of six of these residues abolished cell-cell adhesion (Aricescu et al., 2007, 2008). When all of the residues of the dimer interface are compared to the residues of PCP-2, two differences are observed: Gly¹⁷⁹ in the MAM domain is a serine in PCP-2; and Trp²⁹⁹ in the first FNIII repeat is an isoleucine residue in PCP-2 (numbering of amino acids begins with the signal sequence). Notably, Trp²⁹⁹ was shown to be absolutely essential for mediating cell-cell adhesion in aggregation assays (Aricescu et al., 2007). This suggests that if the hypothesized trans-dimer interface is as predicted, this amino acid change may be sufficient to render PCP-2 unable to mediate cell-cell adhesion. Furthermore, structural comparison of the MAM domain of PTP μ with the ligand-binding domains of EphB2 receptor and laminarinase highlighted two regions, termed L1 and L2, that are also potentially part of the dimer interface (Aricescu et al., 2006). In the L2 loop, two residues required for mediating cell-cell aggregation differ between PTP μ and PCP-2: Pro⁵⁷ is a tyrosine and Pro⁶¹ is a glutamic acid residue in PCP-2 (Aricescu et al., 2006). None of these changes are likely to be the entire answer, however, as these residues are not necessarily conserved in PTP κ or PTP ρ , either. For example, PTP κ has a serine at residue 179, and a leucine at residue 299 yet it is still able to mediate cell-cell aggregation.

Previously, a soluble PCP-2 construct containing the MAM, Ig, and first FNIII repeat was shown to mediate bead aggregation *in vitro* (Cheng et al. 1997). Our study demonstrates that wild-type PCP-2 cannot mediate cell-cell aggregation. As mentioned earlier, the Ig domain of PTP μ alone is sufficient to mediate *in vitro* bead aggregation (Brady-Kalnay and Tonks 1994). Three amino acid residues within the Ig domain of PTP μ were proposed as important in mediating this adhesive interface: Phe¹⁸⁸, and Arg²³⁹ and Arg²⁴⁰ (Aricescu et al. 2007). These three residues are conserved in PCP-2. Furthermore, our domain swapping

experiments demonstrate that the MAM and Ig domains of PCP-2 are both capable of some adhesive function when expressed with functional FNIII domains of PTP μ . Therefore, we hypothesize that PCP-2, like PTP μ , is able to mediate bead aggregation in large part due to its Ig domain. Future studies will aim to identify which residues in PCP-2 render it unable to mediate cell-cell aggregation while still able to mediate *in vitro* bead binding.

PCP-2 is predicted to be a tumor suppressor gene based upon its reduced expression in melanomas (McArdle et al. 2001). PCP-2 regulates the cadherin-catenin complex by interacting with and dephosphorylating β -catenin, thereby promoting E-cadherin-mediated adhesion (Yan et al. 2002; Yan et al. 2006). Because of its inability to mediate cellular adhesion itself, PCP-2 likely functions as a tumor suppressor gene via regulation of tyrosine phosphorylation, potentially of the cadherin-catenin complex.

The high degree of sequence identity in the cytoplasmic domain (68%) between the type IIb RPTPs was predictive in determining the cross-reactivity of the PTP μ cytoplasmic domain antibodies. This is a necessary study, as the antibodies we generated are now commercially available from companies such as Santa Cruz Biotechnologies (SK10 and SK15) and Calbiochem (SK15), and are advertised as PTP μ -specific. While SK6, SK10 and the C-terminal PTP μ antibody are specific to PTP μ , the other cytoplasmic antibodies cross-react with other subfamily members. Future studies utilizing these antibodies will need to take this cross-reactivity into consideration.

In summary, the data presented in this paper supports earlier observations that type IIb RPTPs function in distinct manners to regulate cell adhesion. Studies such as this are important to understand how RPTPs function in cells to gain insight into how alterations in RPTPs function in disease states, such as during tumor progression.

Acknowledgments

We thank Sara Lou for preparation of the figures and graphs, Dr. Scott Howell for assistance with quantitation and Dr. Jan Sap for the gift of the PTPRK plasmid. We thank Samantha Oblander and Polly Phillips-Mason for assistance with sequence lineups as well as Greg Pietz and Yun Qiu for PCP-2 plasmid preparation. This research was supported by grants from the National Institutes of Health Grant R01-CA127590 (Z.W.), R01-NS051520 (S.B.K.). Additional support was obtained from the V foundation, R01-HG004722, HG004722-02S1, U54 CA116867 (Z.W) and the Visual Sciences Research Center Core Grant PO-EY11373 from the National Eye Institute (S.B.K.).

References

- Aricescu AR, Hon WC, Siebold C, Lu W, van der Merwe PA, Jones EY. Molecular analysis of receptor protein tyrosine phosphatase μ -mediated cell adhesion. *EMBO J.* 2006; 25:701–12. [PubMed: 16456543]
- Aricescu AR, Siebold C, Choudhuri K, Chang VT, Lu W, Davis SJ, van der Merwe PA, Jones EY. Structure of a tyrosine phosphatase adhesive interaction reveals a spacer-clamp mechanism. *Science.* 2007; 317:1217–20. [PubMed: 17761881]
- Aricescu AR, Siebold C, Jones EY. Receptor protein tyrosine phosphatase μ : measuring where to stick. *Biochem Soc Trans.* 2008; 36:167–72. [PubMed: 18363557]
- Brady-Kalnay, S. Ig-superfamily phosphatases. In: Sonderegger, P., editor. *Ig Superfamily Molecules in the Nervous System.* Harwood Academic Publishers; Zurich: 1998.
- Brady-Kalnay, SM. Protein tyrosine phosphatases. In: Beckerle, M., editor. *Cell Adhesion: Frontiers in Molecular Biology.* Oxford University Press; Oxford, UK: 2001.
- Brady-Kalnay SM, Flint AJ, Tonks NK. Homophilic binding of PTP μ , a receptor-type protein tyrosine phosphatase, can mediate cell-cell aggregation. *J Cell Biol.* 1993; 122:961–72. [PubMed: 8394372]

- Brady-Kalnay SM, Tonks NK. Identification of the homophilic binding site of the receptor protein tyrosine phosphatase PTP μ . *J Biol Chem*. 1994; 269:28472–7. [PubMed: 7961788]
- Campbell RE, Tour O, Palmer AE, Steinbach PA, Baird GS, Zacharias DA, Tsien RY. A monomeric red fluorescent protein. *Proc Natl Acad Sci U S A*. 2002; 99:7877–82. [PubMed: 12060735]
- Cheng J, Wu K, Armanini M, O'Rourke N, Dowbenko D, Lasky LA. A novel protein-tyrosine phosphatase related to the homotypically adhering κ and μ receptors. *J Biol Chem*. 1997; 272:7264–7277. [PubMed: 9054423]
- Cismasiu VB, Denes SA, Reilander H, Michel H, Szedlacsek SE. The MAM (meprin/A5-protein/PTP μ) domain is a homophilic binding site promoting the lateral dimerization of receptor-like protein-tyrosine phosphatase μ . *J Biol Chem*. 2004; 279:26922–31. [PubMed: 15084579]
- Del Vecchio RL, Tonks NK. The conserved immunoglobulin domain controls the subcellular localization of the homophilic adhesion receptor protein-tyrosine phosphatase μ . *J Biol Chem*. 2005; 280:1603–12. [PubMed: 15491993]
- Duguay D, Foty RA, Steinberg MS. Cadherin-mediated cell adhesion and tissue segregation: qualitative and quantitative determinants. *Dev Biol*. 2003; 253:309–23. [PubMed: 12645933]
- Gebbink MF, van Etten I, Hateboer G, Suijkerbuijk R, Beijersbergen RL, Geurts van Kessel A, Moolenaar WH. Cloning, expression and chromosomal localization of a new putative receptor-like protein tyrosine phosphatase. *FEBS Lett*. 1991; 290:123–30. [PubMed: 1655529]
- Gebbink MF, Zondag GC, Wubbolts RW, Beijersbergen RL, van Etten I, Moolenaar WH. Cell-cell adhesion mediated by a receptor-like protein tyrosine phosphatase. *J Biol Chem*. 1993; 268:16101–4. [PubMed: 8393854]
- Higuchi R, Krummel B, Saiki RK. A general method of in vitro preparation and specific mutagenesis of DNA fragments: study of protein and DNA interactions. *Nucleic Acids Res*. 1988; 16:7351–67. [PubMed: 3045756]
- Lilien J, Balsamlo J. The regulation of cadherin-mediated adhesion by tyrosine phosphorylation. *Curr Opin Cell Biol*. 2005; 17:459–465. [PubMed: 16099633]
- McArdle L, Rafferty M, Maelandsmo GM, Bergin O, Farr CJ, Dervan PA, O'Loughlin S, Herlyn M, Easty DJ. Protein tyrosine phosphatase genes downregulated in melanoma. *J Invest Dermatol*. 2001; 117:1255–60. [PubMed: 11710941]
- Niessen CM, Gumbiner BM. Cadherin-mediated cell sorting not determined by binding or adhesion specificity. *J Cell Biol*. 2002; 156:389–399. [PubMed: 11790800]
- Ostman A, Hellberg C, Bohmer FD. Protein-tyrosine phosphatases and cancer. *Nat Rev Cancer*. 2006; 6:307–20. [PubMed: 16557282]
- Phillips-Mason PJ, Gates TJ, Major DL, Sacks DB, Brady-Kalnay SM. The receptor protein-tyrosine phosphatase PTP μ interacts with IQGAP1. *J Biol Chem*. 2006; 281:4903–4910. [PubMed: 16380380]
- Phillips-Mason PJ, Mourton T, Major DL, Brady-Kalnay SM. BCCIP associates with the receptor protein tyrosine phosphatase PTP μ . *J Cell Biochem*. 2008; 105:1059–72. [PubMed: 18773424]
- Sallee JL, Wittchen ES, Burrige K. Regulation of cell adhesion by protein-tyrosine phosphatases: II. Cell-cell adhesion. *J Biol Chem*. 2006; 281:16189–92. [PubMed: 16497667]
- Sap J, Jiang YP, Friedlander D, Grumet M, Schlessinger J. Receptor tyrosine phosphatase R-PTP- κ mediates homophilic binding. *Mol Cell Biol*. 1994; 14:1–9. [PubMed: 8264577]
- Wang H, Lian Z, Lerch MM, Chen Z, Xie W, Ullrich A. Characterization of PCP-2, a novel receptor protein tyrosine phosphatase of the MAM domain family. *Oncogene*. 1996; 12:2555–62. [PubMed: 8700514]
- Yan HX, He YQ, Dong H, Zhang P, Zeng JZ, Cao HF, Wu MC, Wang HY. Physical and functional interaction between receptor-like protein tyrosine phosphatase PCP-2 and beta-catenin. *Biochemistry*. 2002; 41:15854–60. [PubMed: 12501215]
- Yan HX, Yang W, Zhang R, Chen L, Tang L, Zhai B, Liu SQ, Cao HF, Man XB, Wu HP, et al. Protein-tyrosine phosphatase PCP-2 inhibits beta-catenin signaling and increases E-cadherin-dependent cell adhesion. *J Biol Chem*. 2006; 281:15423–33. [PubMed: 16574648]
- Yu J, Becka S, Zhang P, Zhang X, Brady-Kalnay SM, Wang Z. Tumor-derived extracellular mutations of PTPRT/PTPrho are defective in cell adhesion. *Mol Cancer Res*. 2008; 6:1106–13. [PubMed: 18644975]

- Zhang P, Becka S, Craig SE, Brady-Kalnay SM, Wang Z. Cancer-derived mutations in the fibronectin III repeats of PTPRT/PTPrho inhibit cell-cell aggregation. *Cell Commun Adhesion*. 2010 In Press
Published online on March 16.
- Zondag GC, Koningstein GM, Jiang YP, Sap J, Moolenaar WH, Gebbink MF. Homophilic interactions mediated by receptor tyrosine phosphatases mu and kappa. A critical role for the novel extracellular MAM domain. *J Biol Chem*. 1995; 270:14247–50. [PubMed: 7782276]

Figure 1.

Cross-reactivity of PTP μ intracellular antibodies with other type IIb RPTPs. Sf9 cells infected to express the type IIb RPTPs subfamily members were lysed and analyzed by immunoblotting with the following anti-PTP μ cytoplasmic antibodies: SK6, SK7, SK10, SK15, SK18, and a C-terminal PTP μ antibody (A). Proteins expressed at the cell surface were isolated and immunoblotted with SK7 and SK18 (B). A summary of the cross-reactivity of the SK series of antibodies and the C-terminal PTP μ antibody with type IIb RPTP members is shown in (C).

Figure 2.

Binding of the SK series of PTPμ antibodies is eliminated when specific amino acids are deleted in the cytoplasmic domain of PTPμ fusion proteins. Recombinant PTPμ fusion proteins containing discrete amino acid composition were resolved by SDS-PAGE and immunoblotted with the SK series of antibodies and the C-terminal PTPμ antibody (A). An illustration of the PTPμ recombinant proteins is shown in (B) along with a summary of the epitope binding experiments.

Figure 3. Identification of the epitopes of the SK series of PTP μ antibodies based on amino acid sequence comparison between type IIb RPTPs. SK6 and SK10 (A) are predicted to bind to amino acids 816–914 in the juxtamembrane domain (JMD-W) and are outlined by a black box. SK7 is predicted to bind to the residues outlined by the black box in (B), 850–855 or 868–877, also in JMD-W. SK15 likely recognizes 958–1161 in the first phosphatase domain (PTP1) (C), while SK18 recognizes the residues outlined by the black box in (D) 941–947, also in PTP1. The PTP μ C-terminal antibody is known to bind to residues 1436–1452 (E). Regions of identity between the type IIb RPTP amino acid sequences are shaded gray.

Figure 4.

PCP-2 does not mediate homophilic cell-cell aggregation. Non-adhesive Sf9 cells were infected with baculoviruses expressing different type IIb RPTP subfamily members and allowed to aggregate for 30 minutes prior to imaging. PTP μ , PTP ρ , PTP κ all mediate homophilic cell aggregation, whereas PCP-2 does not.

Figure 5.

PCP-2 does not interact in trans with other type IIb RPTPs to mediate heterophilic cell-cell aggregation. Sf9 cells expressing both GFP and PCP-2 were mixed with Sf9 cells expressing RFP and either PTP μ , PTP ρ or PTP κ . After 30 minutes of aggregation, the cells were imaged. Phase contrast images show that aggregates form. The overlay fluorescent images demonstrate that these aggregates are composed exclusively of RFP expressing cells, and exclude GFP/PCP-2 expressing cells.

Figure 6.

PTP κ expressing cells sort away from PTP μ and PTP ρ expressing cells. Sf9 cells co-infected with RFP and PTP κ were mixed with cells co-infected with GFP and either PTP μ or PTP ρ and subjected to aggregation assay for 30 minutes. A control experiment with Sf9 cells expressing only RFP or GFP demonstrate that these two fluorescent proteins do not induce Sf9 cell aggregation. The overlay images demonstrate that only exclusively red or green aggregates formed in all aggregation experiments, demonstrating that the type IIb RPTPs sort away from each other. Phosphatase expression was detected in Sf9 cells grown in culture by immunocytochemistry with SK15 antibody and a Texas Red secondary antibody. The GFP expression following co-infection is shown in green. The overlay image demonstrates that GFP is co-expressed in the same cells as the phosphatases PCP-2 or PTP μ .

Figure 7.

Expression of PTPp-PCP-2 chimeric proteins on the surface of Sf9 cells. PTPp-PCP-2 chimera proteins were engineered (A) and expressed in Sf9 cells. Within the extracellular segment, the oval represents the MAM domain, the circle with the S-S symbol represents the Ig domain, and the solid rectangles represent the FNIII repeats. In the intracellular segment, the oval represents the juxtamembrane domain, and the aqua shaded rectangles represent the PTP domains. Total cellular protein as well as cell surface expressed proteins were isolated and immunoblotted with SK15 (B). Control expression of actin is shown for the cell lysates (B). There is no actin contamination of the cell surface labeling samples thus serving as a control for the procedure.

Figure 8.

Swapping of either the MAM or Ig domain of PCP-2 into PTP ρ creates a functional protein capable of mediating weak cell-cell aggregation. PTP ρ -PCP-2 MAM and PTP ρ -PCP-2 Ig chimeric proteins are both capable of mediating weak cell-cell aggregation (A), more than PCP-2 but less than wild-type PTP ρ (B). PTP ρ -PCP-2 Extra is not capable of mediating cell-cell aggregation.

Adhesion molecule 1 (green)	Adhesion molecule 2 (red)	Heterophilic binding
PCP-2	PTP μ	–
PCP-2	PTP ρ	–
PCP-2	PTP κ	–
PTP μ	PTP κ	–
PTP ρ	PTP κ	–
PTP μ^*	PTP ρ^*	–
PTP ρ -PCP-2 MAM	PTP ρ	–
PTP ρ -PCP-2 Ig	PTP ρ	–

* This data was presented in our previous publication (Yu et al., 2008)

Figure 9.

Chimeric PTP ρ -PCP-2 proteins sort away from each other. Sf9 cells co-infected with RFP and PTP ρ were mixed with cells co-infected with GFP and the chimeric PCP-2 proteins and evaluated in aggregation assays for 30 minutes. The overlay images demonstrate that only exclusively red or green aggregates formed in these experiments, demonstrating that chimeric proteins sort away from wild-type PTP ρ and create a unique recognition site. A summary table of all heterophilic binding experiments is shown at the bottom of this figure.

Figure 10. Representation of type IIb RPTs using solvent accessible surface charge data. The diagram is contoured from -10kT to 10kT with a red (negative electrostatic potential) to white (neutral charge) to blue (positive electrostatic potential) ramping scheme. The location of the MAM and FNIII-3 domains are indicated to orient the reader. The ribbon diagram illustrates both the trans-dimer interface and the various domains described in this manuscript. The MAM domain is shown as magenta. The Ig domain is shown in green. FNIII repeat 1 is yellow, while FNIII repeat 2 is blue and FNIII repeat 3 is red.