Regulatory Mechanisms for Receptor Protein Tyrosine Phosphatases

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Contents

I. Introduction 2441
II. Regulation of RPTPs by Dimerization 2442
A. CD45: The Prototypical Receptor-Like Protein Tyrosine Phosphatase 2442
B. Negative Regulation of CD45 Function by Dimerization 2442
C. Structural Basis for Inhibition of RPTPα by Dimerization 2443
D. A Model for Negative Regulation of RPTPs by Dimerization 2443
E. Functional Analysis of the Role of the Structural Wedge in Regulating RPTPs 2444
F. Physiological Analysis of the Role of the Structural Wedge in Regulating RPTPs 2445
G. Crystal Structures of Other RPTPs 2445
III. Regulation of RPTP Dimerization 2446
IV. Regulation of RPTPs by Phosphatase Domain 2 2446
A. Functional Requirement for Phosphatase Domain 2 of CD45 2446
B. Protein–Protein Interactions Mediated by Phosphatase Domain 2 2446
V. Regulation of RPTPs by Phosphorylation 2447
VI. Conclusion 2447
VII. References 2447

I. Introduction

Numerous cellular signal transduction pathways utilize protein tyrosine phosphorylation to transmit extracellular stimuli into cellular responses such as proliferation, tissue differentiation, and immune responses.[1] Protein tyrosine phosphorylation is regulated by two families of enzymes: (1) protein tyrosine kinases, which catalyze phosphate transfer, and (2) protein tyrosine phosphatases (PTPs), which catalyze phosphate hydrolysis. The phosphorylation state of a given tyrosine is determined by the balance of specific kinase and phosphatase activities.

All protein tyrosine phosphatases possess at least one catalytic domain of approximately 230−280 amino acids containing the highly conserved active site consensus motif: [I/V]HCXAGXXR[S/T]G.[2,3] Furthermore, the family of protein tyrosine phosphatases can be subdivided into two main classes based on their subcellular localization: (1) intracellular and (2) receptor-like.

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Intracellular PTPs usually possess a single catalytic domain in conjunction with any number of additional protein domains that serve to target the PTP to particular subcellular locations, such as

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nuclear localization sequences, SH2 domains, and PDZ domains.[2−4] Receptor-like PTPs (RPTPs) are integral transmembrane proteins consisting of diverse extracellular domains, a single transmembrane domain, and a cytoplasmic domain usually containing tandemly duplicated phosphatase domains.[2−5] The membrane−proximal phosphatase domain generally accounts for the catalytic activity, while the function of the second domain remains poorly characterized; additionally, there is a subset of RPTPs which possess only a single catalytic domain.

All PTPs utilize the same catalytic mechanism, which employs the cysteine residue in the consensus motif for nucleophilic attack on the substrate phosphotyrosine forming a transient phospho−enzyme intermediate.[6,7] This step is facilitated by an essential aspartic acid residue which serves as a proton donor to the leaving group phenolic oxygen. The reaction is completed through hydrolysis of the phospho−enzyme intermediate, which is facilitated by the same aspartic acid through proton abstraction from a water molecule. Additional amino acid residues are essential for stabilizing the enzyme−substrate interaction. The crystal structures of the intracellular PTP, PTP1B, with and without a substrate peptide, demonstrate that the catalytically essential aspartic acid is located on a flexible, mobile loop.[8,9] In the absence of substrate, this loop is orientated away from the active site; however, upon substrate binding, the mobile loop undergoes a dramatic conformational change, positioning the aspartic acid appropriately within the active site for catalysis to proceed.

Protein tyrosine phosphatases are intrinsically highly active enzymes, with roughly 2−3 orders of magnitude greater activity than that of protein tyrosine kinases.[1] Additionally, PTPs generally display poor substrate specificity in vitro, serving to potently dephosphorylate most phosphotyrosine-containing substrates. Thus, in vivo, PTP activity must be tightly regulated to ensure effective signaling responses. Such regulation can be accomplished in several ways: (1) modulation of steady-state protein levels, (2) alternative mRNA splicing, (3) posttranslational modification, (4) protein−protein interactions, including dimerization, and/or (5) subcellular localization. These mechanisms are applicable to both intracellular PTPs and RPTPs. This review will focus on regulatory mechanisms for receptor-like protein tyrosine phosphatases.

II. Regulation of RPTPs by Dimerization

Dimerization is an important regulatory mechanism for many signal transduction molecules, in particular transmembrane receptor proteins.[10,11] Extensive studies have established that ligand-induced dimerization (or oligomerization) is critical for the activation of receptor tyrosine kinases, antigen receptors, cytokine receptors, TGF-β family receptors, and others. In most cases, dimerization activates receptor kinase activity, either endogenous or associated, by trans-autophosphorylation. RPTPs often possess extracellular motifs common to receptor proteins, but in most cases, ligands have not been identified. In general, the physiological roles and biochemical substrates of most RPTPs are poorly characterized. However, CD45 is one RPTP for whom its physiological roles and biochemical functions are well characterized.

A. CD45: The Prototypical Receptor-Like Protein Tyrosine Phosphatase

CD45 is a RPTP expressed on all nucleated hematopoietic cells, where it is required for signal transduction through antigen receptors.[12−15] The requirement for CD45 has been demonstrated by studies of (1) numerous CD45-deficient T and B cell lines,[16−19] which fail to respond to antigen receptor stimulation, (2) CD45-deficient mice, which show profound blocks in both T and B cell development and function,[20,21] and (3) CD45-deficient humans, who have a severe combined immunodeficiency (SCID) phenotype similar to that observed in CD45-deficient mice.[22,23] At least one function of CD45 is to dephosphorylate the C-terminal site of negative regulatory tyrosine phosphorylation within src-family kinases,[24−29] thereby maintaining them in a "primed" state capable of full activation upon antigen receptor stimulation.[30,31]

Like most members of the RPTP family, CD45 consists of an extracellular domain, a single transmembrane domain, and a cytoplasmic domain containing tandemly duplicated protein tyrosine phosphatase (PTP) domains. The extracellular domain possesses several features consistent with a role in regulating CD45 function. First, CD45 contains multiple fibronectin type III repeats[32] and a cysteine-rich motif found within numerous receptors,[33] which may be involved in protein−protein interactions. Second, CD45 exists as several isoforms due to alternative splicing of exons 4, 5, and 6 within the extracellular domain.[12] The alternatively spliced exons encode multiple sites of O-linked glycosylation so that the extracellular domain of high molecular weight isoforms ($CD45RA^+$) differs in structure and overall charge from the low molecular weight isoform (CD45R0) that lacks these three exons.[34] Resting and activated B cells express the highest molecular weight isoform containing all three exons, referred to as B220 (220 kDa) or CD45RABC. Finally, in T cells, the alternative splicing of CD45 is regulated so that naïve T cells predominantly express $CD45RA^+$ isoforms and switch to expression of CD45R0 (180 kDa) upon activation.[35,36] These observations suggest that the extracellular domain regulates CD45 function, perhaps by binding to a ligand or by mediating dimerization.

B. Negative Regulation of CD45 Function by Dimerization

While much is known about the function of CD45, the manner in which CD45 is regulated (if at all) is not well characterized nor has any ligand been identified for CD45. One early strategy to explore this issue involved examining the functional consequences of treating T cells with anti-CD45 antibodies; some antibodies enhanced T cell signaling, while others were inhibitory.[37−39] While not conclusive, these studies suggest that CD45 may be regulated by

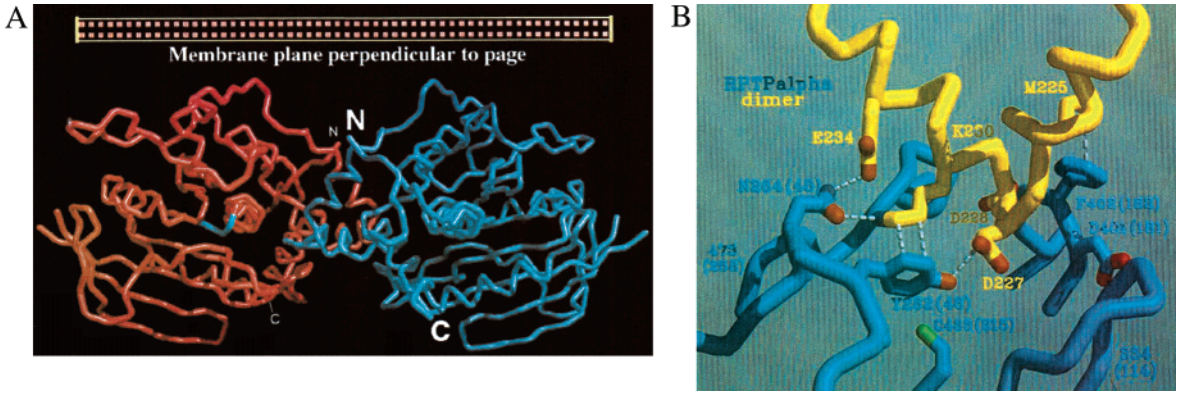

Figure 1. (A) Membrane proximal region and phosphatase domain 1 of RPTPα crystallizes as a dimer. RPTPα is a member of the receptor-like protein tyrosine phosphatase family. This crystal structure demonstrates the formation of a symmetrical dimer in which the phosphatase domain 1 catalytic site of one molecule is blocked by specific contacts with a structural wedge from the membrane−proximal region of the other. Note the wedge of the blue molecule occupying the active site of the red molecule, whose active site cysteine is indicated in blue. (B) Detailed view of the interaction between the structural wedge and the catalytic site in the RPTPα crystal. Hydrogen-bonding interactions are detected between multiple residues in the wedge (yellow) and the opposing catalytic site (blue). Aspartic acid 228 is observed to form a hydrogen bond with the "mobile loop" of the catalytic site; this interaction constrains the loop away from the active site. As PTP catalysis requires this loop to fold into the active site upon substrate binding, this interaction would generate an inactive PTP enzyme. (Reprinted with permission from *Nature* (http://www.nature.com), ref 47. Copyright 1996 Macmillan Magazines Ltd.)

dimerization (or aggregation). Subsequent studies of T cell lines biochemically identified homodimeric forms of CD45 through chemical cross-linking and sucrose gradient centrifugation;[40] however, the manner in which dimer formation is regulated was not determined. Attempts by numerous investigators have failed to definitively identify any ligand for CD45, although it does appear that CD45 makes an adhesive interaction with CD22 on B cells through carbohydrate recognition;[41] however, this interaction does not regulate CD45 function nor is it specific for CD45 as CD22 binds many sialyated glycoproteins.[42] Collectively, these data suggest that CD45 may form dimers in vivo, with functional consequences for signal transduction.

The consequences of dimerization on CD45 function were examined with a chimeric molecule consisting of the extracellular and transmembrane domains of the epidermal growth factor receptor (EGFR) fused to the cytoplasmic domain of CD45 (EGFR−CD45).[43] The EGFR is a receptor tyrosine kinase activated by EGF, its soluble dimerizing ligand. Evidence indicates that only the extracellular and transmembrane domains of the EGFR are required for ligand binding and dimerization.[44−46] Thus, soluble EGF should dimerize the EGFR−CD45 chimeric molecule. Stably transfected EGFR−CD45 restored T cell receptor (TCR)-mediated signal transduction in a CD45-deficient T cell line. Strikingly, treatment of these cells with EGF inhibited TCR-mediated signal transduction, both calcium flux and inducible tyrosine phosphorylation. This inhibition depended on the dimerization of the CD45 cytoplasmic domain as coexpression of a truncated EGFR, consisting of the extracellular and transmembrane domains alone, reversed the effect. Analysis of TCR-induced protein tyrosine phosphorylation in cells inhibited by EGF revealed reduced phosphorylation of the CD3 zeta chain and ZAP-70, indicating a very proximal block in the TCR signal transduction cascade; furthermore, as these molecules are substrates of the src-family kinase Lck, their reduced phosphorylation suggests that Lck activity is inhibited. These data are consistent with dimerization-mediated inhibition of CD45 activity or at least accessibility to its substrate Lck Y505, the C-terminal site of negative regulatory tyrosine phosphorylation. Thus, in contrast to the activating role of dimerization for other receptors, ligand-induced dimerization seems to negatively regulate CD45 function in TCR signal transduction.

C. Structural Basis for Inhibition of RPTPα by Dimerization

RPTPα, another member of the RPTP family, may also be negatively regulated by dimerization as indicated by the crystal structure of its membrane−proximal region and PTP domain 1.[47] The overall structure of the catalytic phosphatase domain is very similar to that observed in the crystal structure of PTP1B. However, unlike PTP1B, the RPTPα fragment crystallized as a dimer. Dimers were observed in two different crystal packing space groups, suggesting that dimerization is not an artifact of crystallization. Significantly, a symmetrical interaction was observed in which the catalytic site of one molecule is blocked by specific contacts with a structural wedge (helix−turn−helix) from the membrane−proximal region of the other (Figure 1A). Not only was the active site sterically occluded, but aspartic acid 228 in the tip of the wedge was observed to make a hydrogen-bonding contact with the mobile loop of the catalytic site, constraining it away from the active site (Figure 1B). As PTP catalysis requires this loop to fold into the active site upon substrate binding, this interaction is predicted to generate an inactive PTP, although phosphatase activity of RPTPα dimers was not reported.

D. A Model for Negative Regulation of RPTPs by Dimerization

Sequence alignment of the analogous membrane proximal region from a subset of RPTPs identified a

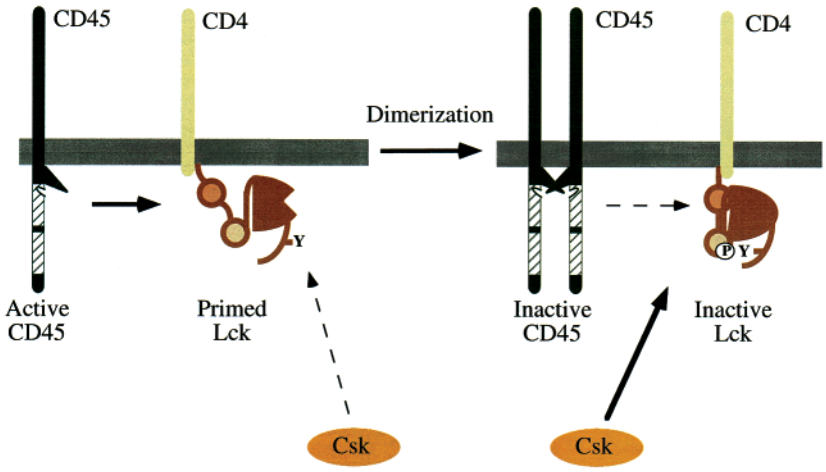

Figure 2. Model for negative regulation of CD45 by the inhibitory wedge. In the monomeric state, CD45 is active and "primes" Lck by dephosphorylating Y505. Dimerization of CD45, by interaction with a ligand or through other means, inactivates CD45 though the symmetrical interactions between the catalytic site of phosphatase domain 1 and the inhibitory structural wedge. Csk activity predominates in phosphorylating Lck Y505, placing it in its inactive conformation and inhibiting T cell signal transduction. The details of this model are depicted here for CD45; however, this mechanism may be more generally applicable to the family of RPTPs.

consensus sequence for the structural wedge; furthermore, database screening with this consensus sequence retrieved all available RPTP domain 1 primary sequences, including CD45, but no RPTP domain 2 or intracellular PTP sequences, suggesting a specific conserved structure and function.[47] Moreover, sequences within this region of CD45 are conserved phylogenetically from shark to human, also implying an important conserved structure and function. Collectively, these observations suggest a model for the regulation of CD45, RPTPα, and other RPTPs, in which dimerization inhibits phosphatase activity, and consequently function, through symmetrical interactions between the catalytic site and the structural wedge (Figure 2).

E. Functional Analysis of the Role of the Structural Wedge in Regulating RPTPs

Given the extensive sequence homology between CD45 and RPTPα in the membrane−proximal region encoding the wedge, the structure of a putative wedge−catalytic site interaction in CD45 was modeled based on the crystal structure of RPTPα (Figure 3). This modeling indicated that it is thermodynamically possible for CD45 to form a similar inhibitory dimer. Furthermore, glutamic acid 624 of CD45, homologous to aspartic acid 228 in the tip of the structural wedge of RPTPα, was observed to make a similar hydrogen-bonding interaction with the mobile loop, constraining it away from the active site. The model for negative regulation of CD45 by dimerization was tested by mutation of this glutamic acid residue in the context of the EGFR−CD45 chimeric molecule.[48] Mutant chimeras were able to stably reconstitute TCR-mediated signaling in CD45-deficient T cells. Treatment of these cells with EGF, however, failed to inhibit TCR-mediated signal transduction. This experiment supports the model by providing evidence of a critical role for the inhibitory wedge in negative regulation of CD45 function by dimerization.

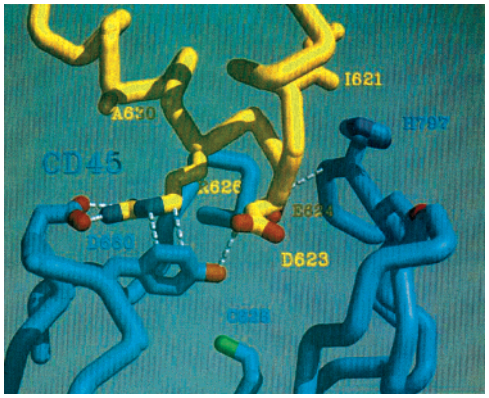

Figure 3. Structural model of the potential wedge/domain 1 interaction in CD45. The structure of a potential wedge/domain 1 interaction in CD45 was modeled based on the crystal structure of RPTPα. CD45 can form a structural wedge, and in dimeric forms of CD45, this wedge can occlude the catalytic site of PTP domain 1 similar to RPTPα. Note that glutamic acid 624 in human CD45 is observed to form a hydrogen bond with the "mobile loop" of the catalytic site constraining the loop away from the catalytic pocket. This interaction would generate an inactive PTP domain 1 of CD45.

Structure−function analysis of CD45 was performed in vitro with purified recombinant proteins.[49] A recombinant protein consisting of the membrane−proximal region and phosphatase domain 1 of CD45 was found to exist primarily as dimers, while recombinant phosphatase domain 2 and the full-length cytoplasmic domain were found to be monomeric. Interestingly, this domain 1 protein fragment was found to be less active than the full-length cytoplasmic domain, even though phosphatase domain 2 had no detectable activity. These observations are consistent with dimerization-mediated inhibition of CD45 activity.

Additional experiments with RPTPα have indicated an essential role for the structural wedge in the negative regulation of RPTPα activity by dimerization. RPTPα is a widely expressed RPTP, with high expression in the brain.[50] RPTPα consists of a

heavily glycosylated short extracellular domain, a single transmembrane domain, and a cytoplasmic domain containing two PTP domains. Evidence is accumulating that RPTPα functions to dephosphorylate the C-terminal site of negative regulatory tyrosine phosphorylation in the Src protein tyrosine kinase, Y529. Dimeric forms of RPTPα were "trapped" through disulfide linkages created by introduction of a cysteine residue immediately N-terminal to the transmembrane domain.[51] Different rotational dimeric interfaces were generated by placing cysteine every two residues over a single turn of an α helix. RPTPα$^{-/-}$ cells were transduced with retroviruses encoding these various forms of RPTPα. Expression of one of four dimeric forms of RPTPα resulted in reduced Src kinase activity and hyperphosphorylation of Y529, indicating inhibition of RPTPα phosphatase activity. The fact that inhibition was observed with only one of four dimers indicates that the manner in which the two molecules are brought together is critical for inhibition. Finally, mutations in the structural wedge of this dimeric RPTPα restored phosphatase activity, supporting the model for negative regulation of RPTPα by dimerization.

F. Physiological Analysis of the Role of the Structural Wedge in Regulating RPTPs

RPTPs are expressed in nonmammalian organisms including *Drosophila*, where five RPTPs have been identified.[5] Genetic analysis has indicated an essential role for several of these RPTPs in motor axon guidance and retinal axon target selection;[52−54] furthermore, mutants of a specific RPTP, PTP69D, are not viable due to unknown developmental defects.[52] PTP69D consists of an extracellular region containing two immunoglobulin-like domains and three fibronectin type III domains, a single transmembrane domain, and a cytoplasmic region with two PTP domains. Structure−function analysis of PTP69D in retinal axon targeting was accomplished through the transgenic introduction of PTP69D mutants into the null background, which indicated that the fibronectin type III repeats and phosphatase activity are required for proper axonal targeting.[55] The requirement for the fibronectin type III repeats indicates that the extracellular domain of PTP69D regulates its function, possibly through interaction with a regulatory ligand or by mediating adhesive interactions. These authors tested the model described above for regulation of RPTPs through the introduction of a PTP69D wedge mutant. This mutant was able to normally reconstitute the axon-targeting defect in PTP69D nulls, suggesting that negative regulation by dimerization does not occur in retinal axon targeting. However, it showed poor rescue of lethality, raising the possibility that PTP69D is normally inhibited by dimerization in other developmental contexts.

The identification of a mutation capable of eliminating negative regulation of CD45 in cultured cells presented the opportunity to test the model in vivo and to simultaneously examine the physiological role of negative regulation of CD45 by dimerization. The experimental approach utilized involved the generation of mice with a germline-targeted mutation inactivating the inhibitory wedge of CD45 (E613R). The model predicts that this mutation will lead to inappropriate CD45 activation under normal dimerizing inhibitory conditions; such dysregulated activity would cause inappropriate src-kinase activation, with potentially pathological consequences. CD45 E613R mice were generated by standard homologous recombination techniques. These mice appeared normal during the first few months of life; however, they subsequently developed a lymphoproliferative syndrome with apparent polyclonal T and B lymphocyte activation and severe autoimmune nephritis with autoantibody production.[56] As a result, these mice died prematurely. The dramatic phenotype of CD45 E613R mice demonstrates the in vivo importance of negative regulation of CD45 by dimerization and, furthermore, strongly supports the model for regulation of CD45 by the structural wedge.

G. Crystal Structures of Other RPTPs

The functional and physiological experiments described above strongly support the model for negative regulation of CD45 and RPTPα by dimerization. However, it is not clear if the structural wedge has an inhibitory function in all RPTPs. The structural wedge was first identified in the dimeric crystal structure of the membrane−proximal region and phosphatase domain 1 (D1) of RPTPα. Alignment of the analogous region from other RPTPs indicated sequence conservation, suggesting a conserved structure. Consistent with this, the structural wedge (helix−turn−helix) is also observed in the two other RPTP crystal structures available, RPTPμ D1,[57] and the cytoplasmic domain of LAR.[58] Thus, it is likely that the wedge is structurally conserved within the family of RPTPs and may also have a conserved function.

It is tempting to speculate that the conserved function of the wedge is to mediate symmetrical inhibition of RPTP dimers. However, the inhibitory interaction between the wedge and the catalytic site detected in the dimeric crystal structure of RPTPα D1 was not observed in the structures of RPTPμ D1 or LAR. In fact, only monomers were detected in both of these structures; the small region of contact observed with RPTPμ D1 was suggested to be an artifact of crystal packing. On this basis, these authors have suggested that inhibitory dimers of RPTPμ D1 and LAR may not form. However, as crystal structures represent only selected conformations of a molecule, these results do not rule out dimerization as a regulatory mechanism for RPTPμ and LAR. In the context of the full-length molecules, dimerization mediated by the extracellular domain could alter the conformation of the cytoplasmic domain, favoring interactions between the structural wedge and catalytic sites. Functional studies will be necessary to determine if these molecules dimerize and if they are negatively regulated by dimerization.

While it is possible that negative regulation by dimerization is restricted to a subset of RPTPs including CD45 and RPTPα, the primary sequence conservation and structural conservation of the wedge within the RPTP family along with the functional

studies of CD45 and RPTPα support a more general model for the negative regulation of RPTPs by dimerization, in which inhibition is mediated by the structural wedge.

III. Regulation of RPTP Dimerization

The discovery that RPTPs can be negatively regulated by dimerization raises the important question of how dimerization of RPTPs is regulated. The extracellular regions of RPTPs often possess a wide array of protein motifs implicated in protein−protein interactions, including immunoglobulin-like domains, fibronectin type III domains, carbonic anhydrase-like domains, cysteine-rich regions, and others, suggesting that these molecules may be regulated by ligands or homotypic aggregation.[4,5] In the case of RPTPα, inactivated homodimers were "trapped" through a disulfide linkage created by introduction of a cysteine residue near the transmembrane domain, suggesting that this RPTP is able to spontaneously dimerize.[51] Recently, biochemical cross-linking experiments have identified cell surface homodimers of RPTPα in transiently transfected cells and mapped the dimeric interaction to multiple regions of the protein.[59] However, the manner in which the RPTPα monomer−dimer transition is regulated is not known. The ectodomains of RPTPμ and RPTPκ were found to mediate homophilic binding interactions,[60−62] but no effect of these interactions on phosphatase activity was detected. Thus, while some protein−protein interactions have been defined for specific RPTPs, in most cases regulatory mechanisms have not been identified.

Multiple ligand interactions have been defined for RPTPβ/ζ, a RPTP expressed in the developing nervous system primarily on glial cells in a pattern suggestive of a function in neuronal migration and axon pathfinding.[63] The extracellular domain of RPTPβ/ζ has been demonstrated to interact with several molecules including contactin, tenascin, multiple cell adhesion molecules (CAMs), pleiotrophin, and others. Interaction of RPTPβ/ζ with these molecules, particularly contactin, affects cell adhesion and neurite outgrowth.[64] Thus, RPTPβ/ζ seems to function as a regulatory ligand for these other molecules. It is also possible that these molecules serve as regulatory ligands for RPTPβ/ζ. Recently, binding of pleiotrophin, a soluble heparin-binding cytokine, to RPTPβ/ζ was found to inhibit the phosphatase activity of RPTPβ/ζ.[65] This is the first demonstration of a soluble, regulatory ligand for any RPTP. Although pleiotrophin-induced RPTPβ/ζ dimerization was not demonstrated, this result is consistent with the model outlined above for regulation of RPTPs by dimerization. Furthermore, this finding increases the likelihood that additional soluble regulatory ligands will be identified for other RPTPs.

IV. Regulation of RPTPs by Phosphatase Domain 2

The majority of RPTPs possess two consensus protein tyrosine phosphatase domains in their cytoplasmic region. However, in most of these proteins the second PTP domain has been found to be inactive; in some cases, one or more of the essential catalytic residues are missing, accounting for the lack of activity. Nonetheless, sequence homology suggests conservation of the architecture of the PTP domain. This was confirmed in the crystal structure of the LAR cytoplasmic domain, in which the structure of phosphatase domain 2 is observed to be similar to that of the catalytically active phosphatase domain 1.[58] These observations suggest that phosphatase domain 2 may be involved in regulation of RPTP function, perhaps by facilitating substrate interactions or by mediating intramolecular interactions.

A. Functional Requirement for Phosphatase Domain 2 of CD45

The requirement for phosphatase domain 2 in RPTP activity and function has been examined most thoroughly for CD45. Early experiments focused on the independent contributions of PTP domain 1 and PTP domain 2 to total CD45 phosphatase activity. Mutation of the essential catalytic cysteine of domain 1 completely eliminated activity of the recombinant protein, while mutation of the catalytic cysteine of domain 2 had no effect on total phosphatase activity, suggesting that only domain 1 is catalytically active.[66,67] In vivo, mutation of the domain 1 cysteine eliminated the ability of CD45 to reconstitute CD45-deficient T cells while mutation of the domain 2 cysteine had no effect.[68] Thus, the essential catalytic function of CD45 is performed by PTP domain 1.

Is phosphatase domain 2 necessary for CD45 activity and function? Initial experiments with recombinant proteins failed to demonstrate phosphatase activity from CD45 PTP domain 1 alone, leading to the suggestion that PTP domain 2 is required for domain 1 to be active.[66] However, recently a recombinant protein consisting of the membrane−proximal region and phosphatase domain 1 has been carefully purified and found to be catalytically active.[49] Thus, it appears that in vitro PTP domain 2 is not required for CD45 catalytic activity. In vivo it has not been possible to determine if domain 2 is absolutely required for CD45 function through expression of molecules lacking domain 2 due to instability of the truncated protein. However, replacement of PTP domain 2 of CD45 with PTP domain 2 of LAR does result in a stable protein, which is catalytically active.[69] When expressed in CD45-deficient T cells, this chimeric protein was unable to reconstitute inducible IL-2 production and ZAP-70 tyrosine phosphorylation. Thus, CD45 phosphatase domain 2 is not required for catalytic activity but is required for CD45 function, suggesting a regulatory role.

B. Protein−Protein Interactions Mediated by Phosphatase Domain 2

One method through which phosphatase domain 2 can regulate RPTP function is by mediating intramolecular or intermolecular protein−protein interactions. Recent analyses of phosphatase domain 2 from several different RPTPs have identified such

interactions. An intramolecular interaction within CD45 was suggested by limited trypsin digestion of a recombinant cytoplasmic domain protein, which produced two large, noncovalently associated fragments.[49] Additionally, recombinant domain 1 (including the wedge) was found to specifically associate with recombinant domain 2 in a GST−fusion protein interaction assay. These authors also determined that the recombinant domain 1 protein formed dimers, suggesting that domain 2 can inhibit domain 1 homodimerization through its own interaction with domain 1, thereby regulating CD45 activity and function.

A direct protein−protein interaction between RPTPα and Grb2 has been demonstrated both in vitro and in vivo.[70,71] This association has been mapped to two separate interactions, the Grb2 SH2 domain with phospho-Y789 of RPTPα (at the C-terminus of phosphatase domain 2) and the Grb2 C-terminal SH3 domain with RPTPα domain 1.[72,73] This second interaction occurs close to the catalytic pocket of phosphatase domain 1, suggesting that it may be involved in inhibiting RPTPα activity. However, the functional consequences of this interaction remain to be determined.

The yeast two-hybrid assay was employed to identify interacting proteins for other RPTPs. In one such screen, the membrane−proximal region and phosphatase domain 1 of RPTPσ was used as bait and identified RPTPδ domain 2 as an interacting protein.[74] This interaction was recapitulated in transiently cotransfected cells. Deletion analysis indicated that the structural wedge of RPTPσ was required for this interaction. Interestingly, this interaction resulted in approximately 50% inhibition of RPTPσ activity using either recombinant or immunoprecipitated proteins. The mechanism of this inhibition is not clear but must be different from that proposed above for domain 1−domain 1 interactions as domain 2 does not contain a structural wedge.

Another yeast two-hybrid screen used the membrane−proximal region of RPTPα as bait and identified RPTPσ domain 2 as an interacting protein.[75] Further analysis indicated that the second domains of RPTPα, LAR, RPTPδ, and RPTPμ all interact with the membrane−proximal region and phosphatase domain 1 of RPTPα. Mutagenesis and deletion analysis indicated that the structural wedge was essential for these interactions. No functional consequences of these interactions were reported for RPTPα. However, as described for CD45, interaction of these RPTP second domains with the wedge of RPTPα may regulate RPTPα by affecting domain 1 homodimerization.

A final yeast two-hybrid screen used phosphatase domain 1 of RPTPμ (not including the wedge) as bait and identified the membrane−proximal region of the same molecule as an interacting protein.[76] Subsequent analysis indicated that this membrane−proximal fragment also interacted with RPTPμ domain 2. Additional experiments suggested that these interactions are intramolecular in both cases, a finding inconsistent with the model proposed for the function of the structural wedge in inhibition upon dimerization. Collectively, these experiments indicate diverse interactions and regulatory mechanisms for the second phosphatase domains of RPTPs, some of which are consistent with the model described above for negative regulation by dimerization and others which may represent novel mechanisms for regulating RPTP activity and function.

V. Regulation of RPTPs by Phosphorylation

Protein modification by phosphorylation is a commonly employed mechanism for regulating enzymatic activity. Experimental evidence indicates that some RPTPs may be regulated by phosphorylation. Treatment of T cells with the calcium ionophore ionomycin led to a decrease in CD45 phosphatase activity that coincided with decreased serine phosphorylation of CD45.[77] Specific phosphorylation sites in CD45 were localized to a 19 amino acid insert within phosphatase domain 2;[78] subsequent reconstitution of CD45-deficient T cells with serine to alanine mutants in these sites resulted in a sustained calcium flux following TCR-stimulation.[79] These results suggest that CD45 can be functionally regulated by serine phosphorylation. Serine phosphorylation may also regulate RPTPα as phorbol-ester treatment of cells induces its serine phosphorylation.[80] Furthermore, this phosphorylation was shown to increase the activity of RPTPα. Mapping of the phosphorylation sites indicated that serine 180 and serine 204 in the membrane−proximal region are substrates of protein kinase C.[81] Notably, these sites are in close proximity to the structural wedge, raising the possibility that phosphorylation disrupts the interaction of the wedge with phosphatase domain 1, resulting in increased phosphatase activity. RPTPα is also phosphorylated on tyrosine at residue 789, creating a binding site for the SH2 domain of the Grb2 adaptor protein.[70,71] Grb2 associates with RPTPα not only through its SH2 domain, but also through an interaction between its C-terminal SH3 domain and a region in RPTPα D1 near the catalytic site, suggesting that this interaction may regulate RPTPα phosphatase activity.[72,73] Future experiments are necessary to evaluate these possibilities.

VI. Conclusion

The numerous experiments described in this review are beginning to reveal the regulatory mechanisms for receptor protein tyrosine phosphatases. Evidence supporting the model for dimerizaton-mediated negative regulation of RPTPs by the structural wedge is accumulating. However, it is not yet clear if this regulatory mechanism can be widely applied to the family of RPTPs. Recent evidence suggests that a subset of RPTPs are regulated in this manner while novel mechanisms may be employed for other RPTPs. These findings provide an excellent basis for future investigation to better understand the regulation of this important family of signal transduction molecules.

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