Multi-ligand interactions with receptor-like protein tyrosine phosphatase β: implications for intercellular signaling

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Receptor-like protein tyrosine phosphatase β (RPTPβ) shows structural and functional similarity to cell adhesion molecules (CAMs). It binds to several neuronal CAMs and extracellular matrix (ECM) proteins that combine to form cell-recognition complexes. Here, the authors discuss the implications of such complexes for intercellular signaling, and the regulation of RPTP activity by cell–cell and cell–ECM contact.

CELL ADHESION MOLECULES (CAMs) from the immunglobulin superfamily modulate cell–cell and cell–ECM (extracellular matrix) interactions that are important for the establishment of intricate networks of connections in the nervous system. These molecules regulate a variety of cellular responses during development, including cell adhesion, cell migration, axonal growth and synaptogenesis; they are also involved in synaptic plasticity in the adult (e.g. structural changes to the synapse that are induced by long-term neuronal activity)[1,2]. CAMs interact with many different ligands and are likely to act in concert by creating cell-recognition complexes. The receptor-like protein tyrosine phosphatases (RPTPs) show structural similarity to CAMs. There is also increasing evidence of functional similarities: like CAMs, certain RPTPs mediate homophilic interactions (an RPTP molecule present on one cell can interact with an identical molecule found on another cell); others interact with recognition molecules and are connected to cytoskeletal proteins. These observations suggest roles for RPTPs in cell–cell interaction and intercellular communication[3].

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The importance of RPTPs during development of the nervous system was recently demonstrated in *Drosophila*. Three axonal RPTPs (DPTP99A, DPTP69D, DALR) were shown to be required for the correct routing and connections of several motor neurons to their target muscles[4,5]. These studies imply that RPTPs might allow growing axons to sense environmental cues; however, they also demonstrate that these RPTPs have partially redundant functions. For example, deletion of a single gene encoding one of the RPTPs has only a subtle effect, but loss of both DPTP99A and DPTP69D results in a much more severe phenotype[5]. A similar degree of redundancy has also been observed in mice lacking individual neuronal CAMs[6]. As with RPTPs in the fly, there is significant overlap in the expression patterns of, and probably also the function of, CAMs: different combinations of CAMs and other membrane-associated molecules are likely to form complexes that transmit signals across the neuronal membrane. This would explain the observation that more than one component must be deleted in order to interrupt transmembrane signaling.

RPTPβ (also known as RPTPζ) expressed on the surface of glial cells binds to the cell-recognition molecule contactin on neuronal cells, leading to neurite outgrowth[7]. Although contactin is a glycosylphosphatidylinositol (GPI)-anchored molecule and lacks a cytoplasmic domain, it can form complexes with at least two transmembrane proteins (the neurexin-like protein Caspr and Nr-CAM) that potentially can transmit signals to the cell interior[8,9]. These interactions form a basis for testing molecular models of intercellular communication during the development of the nervous system. In this article, we will discuss the interactions between glial cells and neurons that are mediated by RPTPβ, contactin and other neuronal cell-surface molecules, and will place special emphasis on the generation and function of cell-recognition complexes.

Receptor tyrosine phosphatase β (RPTPβ)

The RPTPβ molecule is made up of several domains. An N-terminal region with high similarity to the enzyme carbonic anhydrase (CAH) is followed by a fibronectin type III repeat (FNIII), which is in turn linked to a long, extracellular, cysteine-free spacer. The spacer is connected through a transmembrane region to two C-terminal cytoplasmic phosphatase domains[10,11]. Three forms of RPTPβ are generated by alternative splicing: two transmembrane forms and a secreted form (Fig. 1). The secreted form (also known as phosphacan), which consists of the entire extracellular region, and the long receptor form are chondroitin sulfate proteoglycans[12,13]. The short

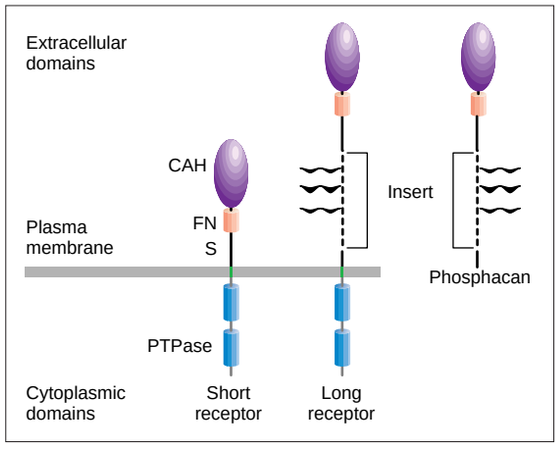

Figure 1
Structure of receptor-like protein tyrosine phosphatase β (RPTPβ) isoforms. All RPTPβ isoforms contain a carbonic anhydrase domain (CAH), a fibronectin type III repeat (FN) and a spacer domain (S). The long receptor form and phosphacan also possess an 860-residue insert that contains glycosaminoglycan side chains (black wavy lines). The receptor forms also contain a transmembrane domain (green) and cytoplasmic tyrosine phosphatase domains (PTPase).

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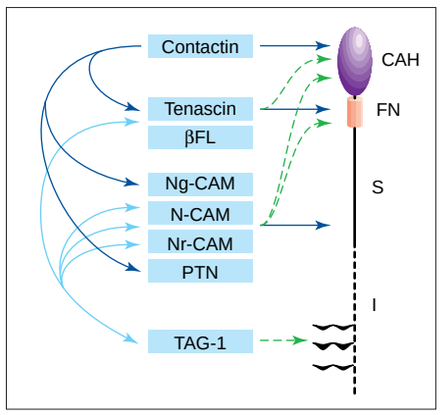

Figure 2

Binding of multiple ligands to the extracellular region of RPTPβ. The carbonic anhydrase domain (CAH) binds to contactin, while the fibronectin repeat (FN) interacts with tenascin and another 220-kDa protein found in glial cells (βFL). The CAH and the FN domains contain asparagine-linked oligosaccharides that mediate the binding of Ng-CAM, N-CAM and tenascin. The spacer domain (S) interacts *in vitro* with several CAMs (including Ng-CAM, N-CAM and Nr-CAM) and with pleiotrophin (PTN). TAG-1, another member of the contactin subfamily interacts with chondroitin sulfate chains (black wavy lines) that are probably located in the 860-residue insert domain (I). Blue arrows represent simple protein–protein interactions and green arrows represent interactions that are mediated by carbohydrates.

receptor form lacks a sequence of 860 amino acid residues, present in the long form, and is mainly detected without glycosaminoglycan[14].

RPTPβ isoforms are found in the developing nervous system primarily on radial glia and astrocytes, in patterns suggesting the involvement of these enzymes in neuronal migration and axonal guidance[15,16]. RPTPβ mRNA is also found in subsets of neurons[17]. The expression of the three forms is developmentally regulated: moderate changes in the levels of the receptor forms and a dramatic increase in the expression of the secreted form occur as development progresses[14]. In general, transmembrane forms of RPTPβ (hereafter the term RPTPβ is used to refer to only the transmembrane forms of the molecule) are found in proliferative zones, while phosphacan is more abundantly distributed throughout the brain. This suggests that the more mature cells, which have migrated out from the neuroepithelium, produce more of the secreted form[15].

The extracellular region of RPTPβ has a complex structure, with multiple domains that interact with a variety of ligands (Fig. 2). Such ligands include the extracellular matrix protein tenascin[18], pleiotrophin, a heparin-binding neurite-promoting factor[19], and several neuronal CAMs from the Ig superfamily[7,20]. Interactions between RPTPβ and CAMs affect cell adhesion and neurite growth. The responses can be either stimulatory or inhibitory, depending on the specific neuronal cell-type studied, and probably reflect the presence of unique recognition complexes in the responding cells. It appears that the short receptor form present on the surface of glial cells promotes neurite growth by interacting with certain CAMs, while the proteoglycan forms can have either inhibitory or repulsive effects[8,20,21]. Contactin interacts with the CAH-like domain in RPTPβ and cooperates with other CAMs that bind to the spacer region (Fig. 2) in promoting neurite growth[7,8]. By contrast, phosphacan might inhibit neurite growth, through an 860-residue insert that has been predicted to be a potent inhibitor of CAM-mediated neural cell growth[20]. Phosphacan could therefore function to regulate cellular interactions by inhibiting the binding of the short receptor form to CAMs and ECM components. Whether the proteoglycan forms can likewise interfere with interactions between the short receptor form of RPTPβ and contactin remains to be seen.

Most of the proteins known to interact with RPTPβ also interact with other recognition molecules, suggesting that they form complexes that modulate cell interactions during development. For example, contactin interacts with Ng-CAM, Nr-CAM and the matrix proteins tenascin and restrictin (see Ref. 22 and references therein). These interactions are mediated by N-terminal Ig-like domains in contactin. The first and second domains are involved in binding to tenascin and Ng-CAM, while the second and third domains mediate interactions with restrictin. The same Ig domains in contactin also mediate its interaction with RPTPβ (Y. Suzuki, E. Peles and J. Schlessinger, unpublished), raising the possibility that RPTPβ/phosphacan regulates CAM action simply by competing for the same binding sites.

Varying the carbohydrate groups attached to surface proteins is another means of regulating cell interaction. Indeed, the sulfation, carbohydrate composition and oligosaccharide structure of phosphacan/RPTPβ are also developmentally regulated, and certain carbohydrates can alter the affinity of RPTPβ for other proteins. The binding of Ng-CAM, N-CAM and tenascin to peptides including the CAH and FNIII domains of phosphacan/RPTPβ has been shown to be mediated by asparagine-linked oligosaccharides[23]. Ng-CAM, N-CAM and Nr-CAM also interact with the spacer region in RPTPβ (Fig. 2), although it is presently unclear whether these interactions are mediated by carbohydrates[8]. In addition, the proteoglycan forms of RPTPβ could interact with other proteins through their chondroitin sulfate chains. Indeed, the sulfated glycans are required for interaction with TAG-1, a member of the contactin subfamily[24].

It was recently reported that pleiotrophin (PTN), a heparin-binding neurite-promoting factor, also binds to phosphacan/RPTPβ[19], and it was suggested that chondroitin sulfate chains on phosphacan/RPTPβ regulate this interaction. These findings raise the possibility that the proteoglycan forms of RPTPβ bind and present growth factors to their receptors in a manner analogous to the way in which heparan sulfate proteoglycans regulate the binding and oligomerization of fibroblast growth factors. However, PTN may also be involved in the regulation of RPTPβ function. RPTPβ also carries the sulfated glucoronic acid that is recognized by the HNK-1 antibody. This carbohydrate epitope was previously found on a variety of cell-recognition molecules, including contactin, Ng-CAM, Nr-CAM and tenascin, and in some cases it has been implicated in interactions between cell-recognition molecules.

Although structurally different from CAMs, RPTPβ mediates similar multifunctional interactions, with different ligands binding to different subdomains of the receptor. These complex interactions have several implications: (1) they might regulate the binding of RPTPβ to contactin; (2) they might control cellular responses to such binding – depending on the ability of particular sets of CAMs acting in concert with contactin in the neuronal membrane to recruit signaling molecules (see below); (3) they might control the submembrane localization of RPTPβ and thereby regulate its enzymatic activity.

Although the effect of the binding of contactin on the intrinsic phosphatase activity of RPTPβ is not yet clear, the recently published structure of the first phosphatase domain of RPTPα raised the interesting possibility that the activity of these enzymes is controlled by dimerization[25]. This study, together with work that utilized chimeras made up of parts of the epidermal-growth-factor receptor and CD45 molecules[26,27], suggests that receptor phosphatases are found in an inactive conformation as dimers and that

dissociation is required for their activation. Accordingly, it is possible that the interaction with contactin, a GPI-linked protein that has high mobility in the plasma membrane, regulates the transition between the monomeric and dimeric forms of RPTPβ, thereby effecting the enzymatic activity of the latter (Fig. 3a, b). The availability of contactin could be controlled by its interaction with other *cis*- and *trans*-acting ligands that are connected to cytoskeletal components. For example, contactin interacts with Nr-CAM. Nr-CAM could localize contactin to specific sites by interacting with ankyrin and possibly with PDZ-domain-containing proteins. Similar localization of contactin might also be controlled by its associated protein, Caspr (see below).

In addition to activation by ligands on opposing cells, it is also possible that ligands that are present in the same membrane as RPTPs regulate their activity in a similar manner (i.e. through lateral interactions; see Fig. 3c, d). In *Drosophila*, the RPTP DPTP10 interacts with a CAM-like protein when they are both expressed in the same cells[28]; it would be interesting to know whether these interactions affect the catalytic activity of the phosphatase. A third mode of regulation could be proposed as an extension of this model: localization of a *cis*-acting ligand by molecules on opposing cells, or in the extracellular matrix, that do not bind directly to the RPTP (Fig. 3e, f). Analysis of the interactions between RPTPβ and contactin will certainly help to determine which (if any) of these possibilities is correct.

Cell-recognition complexes

The action of contactin as a neuronal receptor depends on its cellular context. In association with RPTPβ and Nr-CAM, contactin promotes neurite growth; by contrast, it mediates neuronal repulsion by restrictin[7,8,29,30]. So how does this protein, which is connected to the outer leaflet of the membrane by a GPI anchor, relay signals that induce neurons to extend processes upon contact with RPTPβ? One possibility is that contactin interacts with other molecules present in the membrane, creating a receptor complex. A search for such molecules identified a 190-kDa protein in a contactin-containing complex that binds to RPTPβ[9]. This protein, termed Caspr (for contactin-associated protein), is a transmembrane molecule that shares homology with neurexin. It interacts with contactin when they are both present in the same plane of the membrane, raising the possibility that the two proteins constitute a co-receptor complex.

The cytoplasmic domain of Caspr contains a proline-rich sequence capable of binding to a subset of SH3 domains in signaling proteins. This suggests that it could function as a signaling component of the putative co-receptor complex[9]. The intracellular domain of neurexin IV, a counterpart of Caspr in *Drosophila*, is required for localization of the D4.1-coracle protein, a protein that is essential for the formation of septate junctions[31]. This raises the possibility that, in vertebrates, Caspr interacts with members of the protein 4.1/ERM family that connect the cytoplasmic membrane to the cytoskeletal network[32].

Another protein that interacts with contactin laterally (in the membrane), and is important for neurite extension induced by RPTPβ, is Nr-CAM (see below). Nr-CAM binds to ankyrin, a spectrin-binding protein that links the actin cytoskeleton to the plasma membrane. The interaction between ankyrin and Nr-CAM is regulated by phosphorylation of a tyrosine residue that is conserved in all members of the Nr-CAM family[33]. Moreover, the phosphorylation of this residue inhibits ankyrin binding and regulates the lateral mobility of neurofascin (another Nr-CAM family member) in the plasma membrane. Nr-CAM also contains a potential binding site for PDZ-containing proteins in its C-terminal tail. An additional candidate for mediating signaling through contactin is an as yet uncharacterized ~75-kDa protein that was found in a complex with contactin and the tyrosine kinase c-Fyn[34]. In summary, the cytoplasmic tails of Caspr and Nr-CAM proteins probably recruit PDZ- or SH3-domain-containing proteins and other signaling molecules to specific regions of cell–cell contact, and thereby regulate cytoskeletal changes during neurite outgrowth (Fig. 4a).

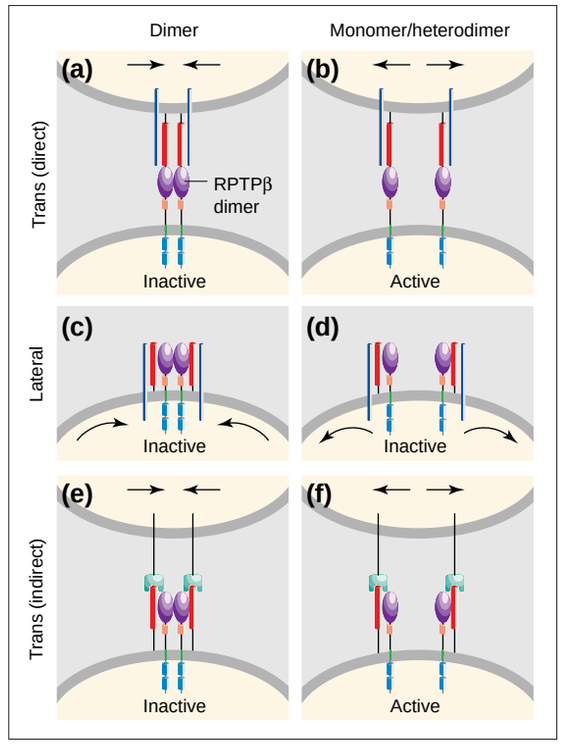

Figure 3

Possible mechanisms for regulation of RPTP activity by ligand-induced dimerization. Dimerization of RPTPs might be regulated by their interactions with various ligands. RPTPs have been proposed to be inactive in dimeric form (a,c,e). Lateral mobility of specific ligands induces formation of monomers and releases the phosphatase from the inhibitory state (b,d,f). Several possible modes of interaction with regulatory ligands are shown. **(a,b)** Direct interaction of the receptor with a ligand (red) present on an opposing cell induces dissociation of the RPTP dimer. As in the case of glycosylphosphatidylinositol-linked ligands, restricted movement in the plasma membrane might be controlled by additional proteins (blue). **(c–f)** Lateral interactions of the phosphatase with a ligand (red) present in the same plane of the cell membrane could also induce dissociation. The movement of the RPTP might be regulated indirectly by other proteins (blue) that bind to the same ligand in the same cell (c,d). Alternatively, proteins in opposing cells (green) may perform this function (e,f).

Specification of signaling pathways

Analysis of neurite growth on different domains of the extracellular region of RPTPβ revealed that in addition to contactin, Nr-CAM is also required for maximal response[8]. This study also indicated that there is cooperation between Nr-CAM and contactin in a hierarchical manner; the interaction between contactin and the CAH domain of RPTPβ is necessary for adhesion but is not sufficient for the full neurite-promoting activity of RPTPβ. Apparently, additional interactions, such as those between the spacer region of RPTPβ and Nr-CAM, are required for the induction of longer neurites. It is possible that adhesion is mediated by contactin and neurite growth by Nr-CAM. However, if this were true, one would expect that once the cells are in contact with RPTPβ, interaction with Nr-CAM would be sufficient to promote outgrowth. This is clearly not the case, because mixing a recombinant spacer region with adhesion molecules did not promote neurite

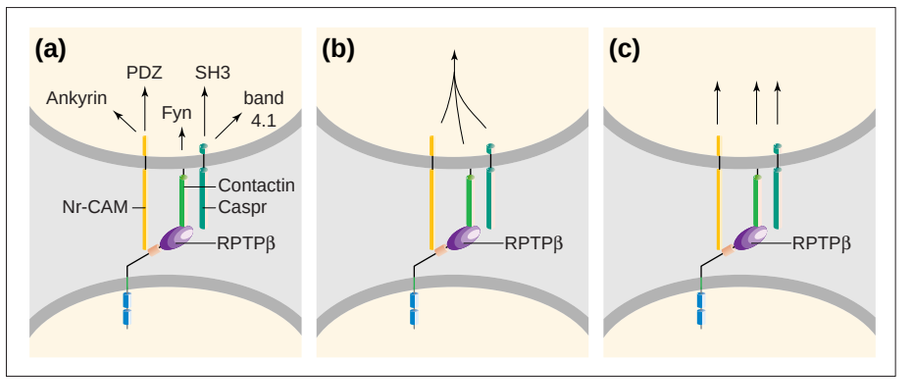

Figure 4

A model for the interactions between RPTPβ and neuronal recognition complexes. RPTPβ interacts with a complex containing contactin, Caspr and/or Nr-CAM. **(a)** Several cytoplasmic proteins, including ankyrin and band 4.1, might connect this recognition complex to the cytoskeleton. Nr-CAM interacts with ankyrin and also contains a consensus binding site for PDZ domains. Caspr interacts with SH3-domain-containing proteins and contains a short sequence that could bind protein 4.1. Maximal induction of neurite growth by RPTPβ requires the involvement of contactin and Nr-CAM in the neuronal membrane. Two possible explanations for this dual receptor requirement are shown in the figure. **(b)** Lateral interactions between these molecules create a functional receptor complex that transmits a unique signal. **(c)** Alternatively, the interactions between RPTPβ and complexes of contactin with Caspr and Nr-CAM might trigger distinct signaling pathways that cooperate to achieve the correct biological response.

growth, whereas mixing the spacer region with a construct containing the CAH and FNIII domains of RPTPβ induced long neurites[8].

Two possible explanations for this dual receptor requirement are depicted in Fig. 4. The first postulates that *cis*-interactions between the two adhesion molecules on the neuronal surface create a functional receptor complex, and that the signal generated depends on the establishment of this specific complex (Fig. 4b). Indeed, it was possible to detect physical association between Nr-CAM and contactin in these cells. Alternatively, the interactions between RPTPβ and complexes of contactin with Caspr and Nr-CAM might trigger different signaling pathways, which cooperate to achieve the correct biological response (Fig. 4c). Recently, it has been shown that heterodimers formed between Ng-CAM and another CAM, Axonin-1, in the axonal membrane are required for promotion of neurite growth of dorsal root ganglia neurons[35]. Interestingly, the interaction between these two CAMs affects their association with protein kinases[36]. During neurite growth (when cells are growing on laminin), Axonin-1 monomers are associated with Src-like tyrosine kinase activity, while Ng-CAM monomers are weakly associated with serine/threonine kinases related to S6 and casein kinases. During fasciculation, when extensive cell–cell contacts are formed, there is increased activity of the casein kinase II associated with Ng-CAM, and the tyrosine kinase activity associated with Axonin-1 disappears. It has been suggested that the casein kinase activity associated with Ng-CAM may regulate cytoskeletal structures[36]. The above findings imply that the association of CAMs in a complex could regulate the recruitment of signaling molecules. It is also possible that different signaling molecules will be utilized by the same CAM during different cellular processes (e.g. neurite growth, fasciculation and adhesion).

Outlook

Studies of the interactions between receptors on the same cell, and between proteins on adjacent cells, are likely to provide key insights into the function of receptor tyrosine phosphatases and cell-recognition molecules in intercellular communication. A major question is that of how cells integrate multiple signals from their environment during development. Understanding how cell-recognition complexes recruit different signaling molecules inside cells, as well as how they modulate extracellular interactions to control neurite growth and guidance, will certainly help to answer this question.

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