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Chapter 5

# The Family of Protein Tyrosine Phosphatases and the Control of Cellular Signaling Responses

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## INTRODUCTION

Every eukaryotic cell is surrounded by a plasma membrane which forms a barrier to the outside world. However, the cell cannot ignore its environment and must be

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able to respond to external stimuli. This is largely achieved through specific proteins that span the membrane. Binding of an effector molecule to the extracellular segment of one of these transmembrane receptor proteins elicits a response in the intracellular milieu. One of the principal features that underlies such signal transduction responses is protein phosphorylation—a reaction catalyzed by protein kinases in which the γ phosphate of ATP is transferred primarily to the hydroxyl groups of seryl, threonyl, or tyrosyl amino acid residues in a target protein. The addition of phosphate alters the conformation of the target protein and thus its function. Therefore, through the activation of a protein kinase, triggering phosphorylation with concomitant modulation of the activity of intracellular target substrates, a cell can be induced to respond to extracellular signals. For example, the transmembrane receptors for many growth factors possess intrinsic protein tyrosine kinase (PTK) activity that is stimulated by ligand binding. In response to ligand the activated receptor PTK then phosphorylates a spectrum of substrates that includes the initiation of a cascade of serine/threonine phosphorylation events, culminating in gene transcription in the nucleus and production of the proteins essential for mediating the growth response. Therefore, growth factor binding initiates a complex network of protein phosphorylation events that lead a quiescent cell to enter the cell cycle, undergo DNA replication and ultimately divide. Similarly, such receptor PTKs are involved in differentiation processes in which a precursor cell is driven to assume a specialized phenotype and to cease dividing, for example, in the generation of neurons. The potential for disruption of such systems to cause disease is obvious. For example, aberrant activation of a PTK, so that the cell is constantly being stimulated to divide in an uncontrolled fashion, has been associated with a number of cancers. The receptor for insulin is also a PTK and defects in its function may contribute to type II diabetes.

Protein phosphorylation, and of particular interest, protein tyrosine phosphorylation, plays crucial roles in the control of fundamental cellular processes including growth, division, and differentiation. However, before we can begin to pinpoint dysfunctional situations in disease states, we must first understand how these various signaling responses are controlled in normal cells. Much research effort has focussed on this problem from the perspective of the protein kinases. However, in my laboratory we are taking a different approach. It is important to realize that within the cell phosphorylation is not static; it is a reversible dynamic process. Thus, the net level of phosphate in a target substrate reflects the balance between the competing action of the kinase that phosphorylates it and enzymes that catalyze the reverse reaction, the protein phosphatases (Figure 1). In my lab we focus on defining physiological roles for tyrosine phosphorylation but from the perspective of the enzymes that catalyze dephosphorylation, that is, the protein tyrosine phosphatases (PTPases).

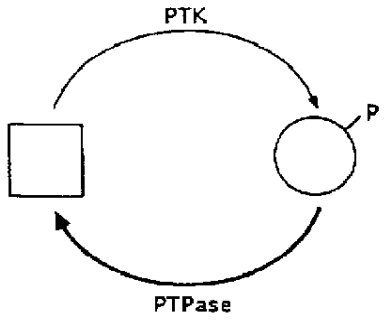


*Figure 1.* Protein phosphorylation is a reversible process *in vivo*. It is important to realize that the net level of phosphate in a target substrate reflects the balance between the competing action of the kinase that phosphorylates it and the phosphatase that catalyzes the dephosphorylation reaction. This chapter addresses the physiological functions of members of the PTPase family.

## STRUCTURAL DIVERSITY IN THE PTPASE FAMILY

Early in the study of the control of glycogen phosphorylase by reversible serine phosphorylation, it was proposed that the major regulation was exerted at the level of the kinase. From there the idea that protein dephosphorylation was catalyzed by a small number of protein phosphatases, serving as constitutively active housekeeping enzymes, pervaded the whole field. However, nothing could be further from the truth. Since protein tyrosine phosphorylation was first observed over 10 years ago, considerable progress has been made in characterizing the PTKs (reviewed in Fantl et al., 1993). The study of PTPases, until recently, lagged significantly behind largely for technical reasons. A major problem in the study of protein phosphatases in general is the requirement for a suitably purified and phosphorylated substrate to measure enzyme activity. In the early days of tyrosine phosphorylation this issue was compounded by the fact that physiologically relevant phosphotyrosyl-containing proteins were largely unidentified. Hence artificial substrates had to be generated. One such protein, reduced carboxyamidomethylated and maleylated (RCM) lysozyme was developed for the assay of PTPase activity *in vitro* and then used to complete the first purification to homogeneity of a novel, phosphotyrosyl-specific protein phosphatase (Tonks et al., 1988b,c). An enzyme termed PTP1B was isolated in homogeneous form. The amino acid sequence of PTP1B was determined and homology with CD45, a transmembrane glycoprotein that is a surface marker of hematopoietic cells was demonstrated (Charbonneau et al., 1988). This result was important because of the structure of CD45. It possesses a tandem array of two PTPase domains in its intracellular segment, a single transmembrane domain and a highly glycosylated, cysteine-rich extracellular segment that displays the hall-

marks of a ligand binding motif. Thus, the exciting possibility was raised that CD45, may represent a prototype for receptor-linked PTPases with the capacity to play a direct role in modulating cellular signaling responses. This was further strengthened by the demonstration of intrinsic PTPase activity in CD45 (Tonks et al., 1988). What followed has been an explosion of interest in the PTPases and now a wide variety of these enzymes have been identified in sources as diverse as mammals, *Drosophila*, *C. elegans*, *Dictyostelium*, yeast, and viruses (reviewed in Charbonneau and Tonks, 1992). PTPases have even been detected in prokaryotes. In fact, it has recently been shown that the essential virulence determinant of the bacterium *Yersinia*, the causative agent of the plague or Black Death, is a PTPase termed Yop, the activity of which is essential for virulence. Apparently, following infection the Yop PTPase dephosphorylates tyrosyl residues in proteins of the eukaryotic host cell, thus disrupting normal cell function (reviewed in Guan and Dixon, 1993)

## STRUCTURAL FEATURES OF THE PTPASE FAMILY

We know now that the PTPases rival the PTKs in their structural diversity and complexity. Each PTPase contains at least one conserved segment of ~240 residues which is assumed to delineate an independently folding catalytic domain. Within this domain is the sequence motif [I/V]HCXAGXXR[S/T]G (where X can be any amino acid) that uniquely defines the PTPase family of enzymes. The cysteinyl residue, which is absolutely conserved, is involved in the formation of a thiophosphate covalent intermediate as part of the catalytic mechanism. Although the PTPases display a high degree of similarity in their catalytic domains, they can be readily differentiated on the basis of the structure of their noncatalytic segments. Most strikingly, like the PTKs, the PTPases can be subdivided into receptor-like and nontransmembrane isoforms. The observation of receptor-like PTPases is important because it predicts a potential to convert extracellular signals directly into intracellular responses through extracellular ligand-mediated dephosphorylation of intracellular target proteins.

## RECEPTOR-LIKE TRANSMEMBRANE PTPASES

The distinguishing features of the receptor-like PTPases include the presence of a single transmembrane segment and a tandem array of two PTPase domains within the cytoplasmic tail. Thus far, six receptor-like PTPases have been found to contain a single catalytic domain the prototype being HPTPβ, however these remain the minority. The significance of the two-domain arrangement that characterizes the receptor-PTPases is unclear. Although there is the obvious potential for cooperative interactions between domains, it remains a point of controversy as to whether in fact both domains, in particular domain II, are active. In some cases it has been proposed that the function of domain II is to control the specificity of domain I. Nevertheless, it seems clear that, at least for CD45 and PTPα, domains I and II both

possess intrinsic activity, but differ in their substrate specificity and possibly in their mode of regulation.

In contrast to the similarity of their intracellular domains, the receptor-like PTPases can be readily distinguished by their extracellular segments. They may be arbitrarily divided into five subtypes on this basis (Figure 2). One might anticipate that the variation in the extracellular segments of these enzymes may reflect a corresponding diversity in the ligands to which they respond. Until recently, a function had not been ascribed to the extracellular segment of any one of these enzymes, thus they remained putative receptors. It was originally suggested that a B-cell surface protein, CD22, interacted with the smallest, 180 kDa form of CD45. However, subsequently it has become clear that CD22 is a lectin that binds generally to sialoglycoproteins including all forms of CD45. The significance of this association remains unclear, although recent observations suggest that binding of CD22 to CD45 amplifies (i.e. positively regulates) early signals through the T cell receptor (Sgroi et al., 1995). In addition, it was demonstrated recently that the

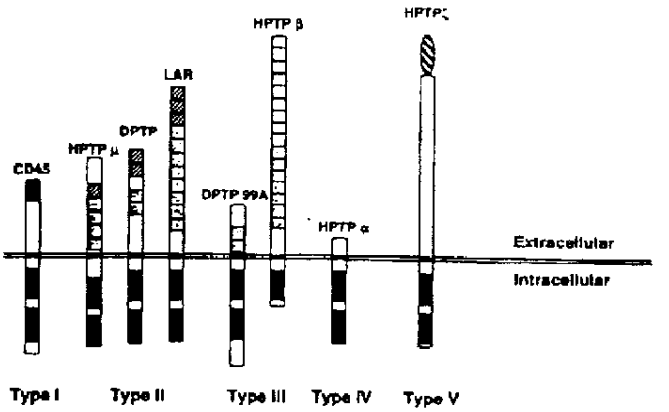


***Figure 2.*** Representatives of the receptor-like PTPases. The conserved catalytic domains are shown in black. At the present time the receptor-like species can be subdivided into five types based on the structure of their extracellular segments. Type I represents the CD45 family, multiple isoforms of which arise from differential splicing of a primary mRNA transcript of a single gene; three exons encoding sequences at the extreme N-terminus (horizontal lines) are differentially expressed. Type II contain immunoglobulin-like (diagonal lines) and tandem fibronectin type III-like repeat domains (stippled); this category includes among others LAR (leukocyte common antigen-related), DLAR, DPTP, and HPTPμ. Type III bear multiple fibronectin type III-like repeats. Some type III isoform, such as HPTPβ, have only one internal PTP domain. Type IV isoforms such as α and ε have small glycosylated extracellular segments. Type V possesses an N-terminal motif with homology to carbonic anhydrase.

carbonic anhydrase-like domain in the extracellular segment of PTPζ/β on glial cells binds specifically to contactin, a glyosylphosphatidylinositol-anchored cell recognition protein on the surface of neurons. Interaction between this domain of PTPζ/β and contactin promotes neuronal adhesion and the induction of neurite outgrowth and differentiation, suggesting that such interactions may underlie the triggering of unidirectional or bidirectional signaling responses between neurons and glial cells during development (Peles et al., 1995).

We have approached this issue of ligands for one of the type II receptor PTPases, PTPμ, and have now demonstrated that this enzyme has the potential to function in cell-cell adhesion reactions (Brady-Kalnay et al., 1993). The extracellular segment of PTPμ contains one immunoglobulin (Ig)-like and four fibronectin (FN) type III-like domains, and thus displays structural similarity to members of the Ig superfamily of cell adhesion molecules that includes NCAM, the neural cell adhesion molecule. NCAM mediates homophilic adhesion, i.e., NCAM on one cell binds to NCAM on an adjacent cell (see Edelman & Crossin, 1991 for review). To investigate whether PTPμ could also serve in cell-cell adhesion, we have followed the strategy of expressing the protein in normally nonadhesive cells to test whether this induces their aggregation. We have generated recombinant baculoviruses that express various forms of PTPμ in Sf9 insect cells and have demonstrated that expression of full length PTPμ, or mutants bearing an intact extracellular segment, whether in the presence or absence of PTPase domains, induced aggregation. However, expression of the catalytic segment of PTPμ as a soluble protein or in a chimeric molecule fused to the extracellular and transmembrane domains of the EGF receptor was ineffective in inducing aggregation. The degree of aggregation of Sf9 cells induced by PTPμ expression is very similar to that observed following expression of other well characterized adhesion molecules in various model systems. Furthermore, we have shown that PTPμ mediates aggregation via a homophilic mechanism. But perhaps most importantly, we have also shown that PTPμ as it is normally expressed on the surface of a lung cell line (MvLu cells), retains the capacity for homophilic binding interactions. Thus, these results suggest that the ligand for this transmembrane PTPase is another PTPμ molecule on the surface of an adjacent cell and provided the first indication of the function of the extracellular segment of one of these enzymes.

The precise physiological role of such binding interactions remains to be defined but it is tempting to speculate that PTPases such as PTPμ may be involved in the control of cell proliferation. As cells grow and divide in culture, they spread out over the surface of the petri dish until eventually forming a uniform, or confluent, layer in which adjacent cells are touching each other. In normal cells this promotes a response termed "contact inhibition of cell growth." When one considers that activation of receptor PTKs, triggering a tyrosine phosphorylation response, can promote cell growth, perhaps activation of a PTPase, triggering tyrosine dephosphorylation, contributes to the mechanism for such growth inhibitory phenomena.

Homophilic association between extracellular segments of receptor PTPases, such as PTPμ, brought into contact as adjacent cells touch in a confluent monolayer, may promote such tyrosine dephosphorylation events. Whether PTPμ can itself drive cell-cell association at its normal levels of expression, i.e., function as an adhesion molecule under physiological conditions, remains unclear. Perhaps cell-cell association facilitated by other adhesion molecules serves to juxtapose the extracellular segments of PTPμ molecules for binding. Furthermore, to date, no direct effect of ligand binding to the extracellular segment of PTPμ on the activity of the intracellular PTPase domains has been detected. Nevertheless, such homophilic binding interactions could serve a tethering role, controlling the activity of the PTPase indirectly by restricting its spatial distribution on the membrane, and thus restricting the spectrum of substrates with which it may interact, and perhaps bringing it into close proximity with an appropriate target. A structural feature that characterizes PTPμ may be important in this regard; its juxtamembrane domain (that between the transmembrane and first PTPase domains) is longer than the equivalent segment in other receptor PTPases and displays homology to the intracellular segments of members of the cadherin family of cell adhesion molecules. The intracellular segment of the cadherins, which is essential for their adhesive function, interacts indirectly with cortical actin through the binding of proteins termed catenins. Thus, the association of the cadherin cytoplasmic domain, the domain that displays homology to PTPμ, with catenins is essential for both the cytoskeletal binding and adhesive function of cadherins. We are currently testing whether protein:protein interactions mediated by this segment in PTPμ serve to target the phosphatase to particular cytoskeletal structures. Most recently we have obtained evidence to indicate that in a variety of tissues and cell lines PTPμ is present in adherens junctions in a complex with cadherins and catenins (Brady-Kalnay et al., 1995). Our data suggest that within these structures PTPμ may be one of the enzymes that regulates the dynamic tyrosine phosphorylation and thus the function of the cadherin/catenin complex *in vivo*. Therefore rather than functioning as an adhesion molecule itself, the physiological role of PTPμ may be to regulate other adhesion systems in response to homophilic binding. In transformed, i.e., cancerous, cells the contact inhibitory response breaks down and cells grow and divide essentially without restraint. It will be of interest to determine whether alterations in PTPases such as PTPμ may contribute to the transformed phenotype.

## CYTOPLASMIC NONTRANSMEMBRANE PTPASES

Unlike their receptor-like counterparts, nonreceptor PTPases have a single catalytic domain and unrelated, noncatalytic sequences of variable length at either their N- or C-termini (Figure 3). Sequence similarities to other well characterized proteins and, in some cases experimental evidence, suggest that one of the primary functions of these noncatalytic segments may be to control enzyme activity. The concept that a major factor in controlling PTPase activity may be to restrict enzyme location to

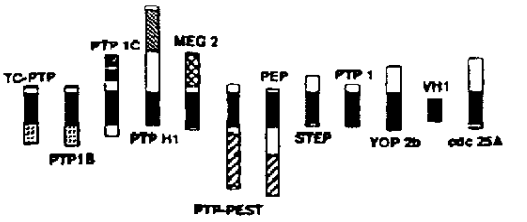


***Figure 3.*** Representatives of the nontransmembrane/cytoplasmic PTPases. The conserved catalytic domains are shown in black. Many of the nonreceptor PTPases bear noncatalytic segments that are structurally related to other well characterized proteins. The position and relative size of these noncatalytic domains are shown as boxes containing distinct symbols; noncatalytic regions that have similar sequences are designated with identical patterns. The noncatalytic segments that have been identified include two SH2 domains in PTP1C, and band 4.1 homology domains in PTPH1, an apparent lipid-binding domain in MEG2, and segments containing PEST sequences in PEP and PTP-PEST. In TC-PTP and PTP1B, the C-terminal noncatalytic segments appear to play a role in modulating activity and controlling subcellular localization. PTP1, STEP, and Yop2b have noncatalytic sequences that are apparently unrelated to sequences in the databases. The protein from *Vaccinia* virus, VH1, is much smaller than the other PTPs and presumably encodes only essential sequences within the catalytic domain. VH1 differs from the other members of the family in that it displays dual specificity, dephosphorylating PSer as well as PTyr.

particular regions of the cell is currently gaining ground. An example of this targeting concept is provided by PTPH1 (Yang and Tonks, 1991). The cDNA for PTPH1 predicts a protein with a large noncatalytic N-terminal segment containing a domain of ~320 residues that displays homology to the superfamily of proteins that includes band 4.1, talin, ezrin, moesin, and the product of the tumor suppressor gene that causes type 2 neurofibromatosis. The presence of this domain apparently defines a family of proteins that are targeted to interfaces between the plasma membrane and the actin cytoskeleton, suggesting that the subcellular location of the PTPase will be similarly restricted. In addition, two closely related but distinct PTPases, one restricted to hematopoietic cells, and the other expressed ubiquitously, have been identified that bear an N-terminal segment containing two SH2 domains (see Neel, 1993). These domains comprise ~100 amino acid residues and are found in a number of proteins that are involved in mediating early cellular effects of growth factor stimulation (Pawson and Schlessinger, 1993). SH2 domains bind to phosphotyrosyl residues in sequence specific contexts. In the case of these PTPases, the presence of the SH2 domains leads to their recruitment into receptor PTK signaling complexes in a manner that is dependent upon ligand binding and receptor autophosphorylation. There is evidence to indicate that, once bound to the receptor PTK, the PTPase becomes activated and presumably it then dephosphorylates other members of the signaling complex whose identity remains to be

established. Also these PTPases are themselves phosphorylated on tyrosyl residues in response to ligand-dependent activation of receptor PTKs. Whether this phosphorylation is part of the activation mechanism or even creates new docking sites for protein:protein interactions among signaling molecules awaits elucidation.

There are also instances of cytoplasmic PTPases in which the noncatalytic segments do not display homology to known motifs but their structure nonetheless implies a targeting function. For example, in PTP1B there is a C-terminal extension of ~120 residues following the catalytic domain that serves a regulatory function. Analysis of the distribution of charged and hydrophobic residues in this sequence illustrates a predominantly hydrophilic segment of 80–90 residues followed by a highly hydrophobic segment, with a hydrophobic index close to that observed for transmembrane domains in receptor proteins, comprising the extreme carboxy-terminal ~35 residues. This hydrophobic stretch of residues has been shown to be both necessary and sufficient for targeting PTP1B to the cytoplasmic face of membranes of the endoplasmic reticulum (Frangioni et al., 1992). A closely related enzyme termed TCPTP, which displays a similar distribution of charged and hydrophobic residues in its regulatory C-terminal segment (although the absolute sequence identity with this segment in PTP1B is only ~20%), is also targeted to the membranes of the endoplasmic reticulum. There are even cases where alternative splicing of a PTPase transcript may generate forms of the enzyme that contain the same catalytic domain but which bear different targeting sequences and thus display different subcellular locations. For example, DPTP61F from *Drosophila* undergoes alternative splicing to generate two nontransmembrane PTPases, each of ~61 kDa, that differ in their extreme C-termini. One displays a highly hydrophobic C-terminus and is found associated with cytoplasmic membranes, while the other bears a segment rich in basic residues, conforming to a classic nuclear localization signal, and is found exclusively in the nucleus (McLaughlin and Dixon, 1993). Similar data, in which alternatively spliced forms display different subcellular locations, have now also been obtained for TCPTP (Lorenzen et al., 1995).

## FURTHER ASPECTS OF THE REGULATION OF PTPASE ACTIVITY

Initial measurements of the activity of PTP1B and CD45 indicated a very high Vmax, 10–1,000-fold in excess of the PTKs *in vitro* (Tonks et al., 1988c, 1990). Thus, the PTPases have the potential to represent a formidable barrier to the action of PTKs *in vivo*. This suggests that mechanisms must exist to control PTPase activity so as to permit the normal function of PTKs. The importance of harnessing PTPase activity *in vivo* has been vividly demonstrated by experiments in which the cytoplasmic enzyme TCPTP was overexpressed in BHK cells (Cool et al., 1992). Overexpression of the full length 48 kDa form of the enzyme, with an intact targeting, regulatory C-terminal segment, did not produce an obvious phenotype. In contrast, overexpression of a truncated 37 kDa form of the enzyme, from which

the regulatory segment had been deleted and which was now constitutively active and no longer targeted to intracellular membranes, produced a catastrophic effect on the cells. Only a small number of cell lines overexpressing the enzyme were obtained and these displayed a multinucleated phenotype, in which nuclear division was asynchronous. This results from a defect in cytokinesis. Clearly, an uncontrolled PTPase can play havoc in the cell, stressing the importance of characterizing and understanding these control mechanisms.

As discussed above many PTPases possess structural motifs suggestive of regulation through intracellular targeting. Also for the receptor-like enzymes there is the potential for modulation of activity by ligand binding. A further tier to the regulation of PTPase activity is the potential for control by reversible phosphorylation of the enzymes themselves. Alteration in the concentration of intracellular free $Ca^{2+}$ by treatment of T-cells with ionomycin leads to a decrease in the phosphorylation of CD45 coincident with a decrease in its PTPase activity (Ostergaard and Trowbridge, 1991). More recently, it has been demonstrated that PTP1B is subjected to multisite phosphorylation *in vivo*, being a point of convergence for the action of at least three distinct Ser/Thr kinases, including PKC and $p34^{cdc2}$ (Flint et al., 1993). The sites of phosphorylation are found in the hydrophilic portion of the regulatory C-terminal segment of PTP1B. A complex reorganization of phosphate within the molecule accompanies the transition into mitosis in HeLa cells. These and other examples point to the importance of phosphorylation as a regulatory device for the control of PTPase activity. For both the receptor-like and nontransmembrane enzymes, phosphorylation and control of intracellular location, regulating activity indirectly by restricting subcellular distribution, are therefore important concepts that apply to the control of PTPase activity *in vivo*. Such protein:protein interactions may not only illustrate new tiers of control of cellular phosphotyrosine levels and thus signal transduction pathways, but may also eventually point to new families of potential oncogenes and anti-oncogenes.

## PHYSIOLOGICAL ROLES FOR PTPases: A ROLE FOR THESE ENZYMES AS NEGATIVE EFFECTORS OF SIGNAL TRANSDUCTION *IN VIVO*

The initial characterization of several PTPases highlighted the fact that their specific activity *in vitro* far exceeded that of the PTKs. This implied that the PTPases may exert a dominant influence on PTKs *in vivo* (Figure 4). This idea was reinforced by the observation that treatment of NRK-1 cells with vanadate, an inhibitor of PTPases, produced enhanced levels of phosphotyrosine and the generation of a transformed morphology (Klarlund, 1985). These observations led to the hypothesis that some PTPases may be the products of tumor suppressor genes, i.e., their deletion or mutation may contribute to the elevation in phosphotyrosine levels that is associated with certain neoplasias. As expected, overexpression of certain PTPases has been shown to suppress or even revert the transformed

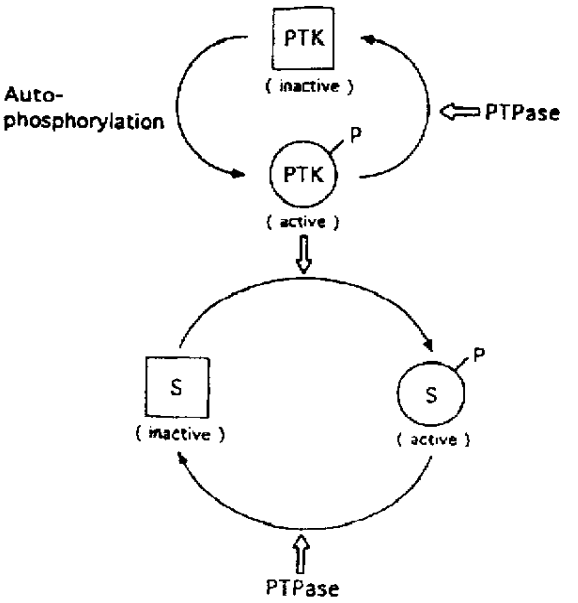


***Figure 4.*** PTPases may act negatively to antagonize PTK-induced signaling events *in vivo*. Since PTKs catalyze the phosphorylation of tyrosyl residues in proteins and PTPases the dephosphorylation of such residues, one can easily see that these enzymes may play antagonistic roles. Many PTKs require autophosphorylation of tyrosyl residues in the kinase itself for optimal activity. Therefore, one could envisage that an inhibitory effect of PTPase may be exerted through the dephosphorylation and inactivation of either the PTK or its target substrate. Reproduced from Sun and Tonks (1994).

phenotype associated with oncogenic PTKs such as Fms, Neu, or Src (see Cool and Fischer, 1993). The notion of PTPases as tumor suppressor genes has triggered a considerable research effort on the part of many groups to examine the possibility of coincidence between the chromosomal localization of various PTPase genes and sites of abnormality associated with cancer. At the present time some leads look promising, in particular, the case of RPTPγ (LaForgia et al., 1991). RPTPγ is a receptor-like PTPase characterized by the presence of an N-terminal segment of ~270 residues that displays homology to carbonic anhydrase. However, only one of the three His residues that are involved in ligating the catalytically essential $Zn^{2+}$ ion in carbonic anhydrase is conserved. Therefore, it has been suggested that rather than catalyzing the hydration of $CO_2$, this carbonic anhydrase-like domain in RPTPγ may function as a hydrophobic binding pocket for a low $M_r$ ligand. The gene for RPTPγ is located on human chromosome 3p21, a region that is frequently deleted in renal and lung carcinomas. A more detailed characterization of the

RPTPγ gene in 31 human lung cancer cell lines did not detect any abnormalities in the segment encoding the intracellular catalytic domains. However, a homozygous deletion has been detected within the RPTPγ gene in all derivatives of murine B cells, a connective tissue line which produces sarcomas in syngeneic mice. This deletion removes residues 12–145 from the full length protein and encompasses a segment of the potential ligand-binding carbonic anhydrase domain (Wary et al., 1993). This shorter transcript has been detected in L cell mRNA. Thus, the intriguing possibility exists that in certain tumors a truncated form of RPTPγ may be expressed which, although it still possesses functional PTPase domains, may not be able to respond normally to its cognate ligand and thus may not trigger a normal signaling response.

Recent studies have highlighted a growth suppressive function for a cytoplasmic PTPase, the SH2 domain-containing PTPase that is expressed exclusively in hematopoietic cells. This enzyme has been given several names, depending upon the group working on it, including HCP, PTP1C, SHP, and SHPTP-1. Mutations in the gene for HCP which result in aberrant splicing of the transcript have been shown to be the cause of the *motheaten* (*me*) phenotype in mice (Shultz et al., 1993). Homozygous *me* mice display severe immunodeficiency and systemic autoimmune disease, and generally only live for 2–3 weeks. In these mice there is a single base deletion in the HCP gene that results in the production of a severely truncated polypeptide of 102 amino acids that is completely devoid of the PTPase domain and most of the SH2 domain sequences. There is a less severe form of the disease, termed *motheaten viable* (*me*$^v$), in which the animals survive for a few months. This results from a distinct mutation that generates protein with sequences either inserted into or deleted from the catalytic domain. This severely impairs catalytic function so that the PTPase activity of *me*$^v$ mutant HCP is reduced by −80% relative to wild type. The broad spectrum of hematopoietic abnormalities associated with the *motheaten* phenotype suggests that HCP may be a negative regulator of several PTK signaling pathways. For example, there is hyperproliferation of macrophages in these mice that is independent of CSF-1. Therefore, one might anticipate that under normal conditions HCP can exert a negative influence on signaling pathways initiated by the CSF-1 receptor PTK. As might be expected, lysates of macrophages from *motheaten* mice display an increase in tyrosine phosphorylation of several proteins relative to the wild type. In addition it has been demonstrated that HCP/SH-PTP1 plays a major role in downregulation of signaling through the erythropoietin receptor (EPOR) (Klingmüller et al., 1995). HCP/SH-PTP1 binds to a site of tyrosine phosphorylation in the intracellular segment of the EPOR (Y429) through its SH2 domains and once recruited into the complex it dephosphorylates and inactivates the JAK2 PTK that associates with the EPOR and is responsible for transmitting the EPO signal. Following expression of Y429F mutant EPORs, in which the docking site for HCP/SH-PTP1 has been converted to phenylalanine and which can no longer bind the phosphatase, cells become hyper-

sensitive to EPO and display prolonged EPO-induced autophosphorylation and activation of JAK2. Thus in general the loss of HCP most likely leads to sustained tyrosine phosphorylation with consequent enhanced proliferation, in a manner analogous to that observed with constitutively activated oncogenic PTKs. The possibility that HCP may contribute to human disease, perhaps also being a tumor suppressor gene, is currently under investigation. It is interesting to note that deletions and translocations of human chromosome 12p12-13, the map position of the HCP gene, are found in patients with acute lymphocytic leukemia.

## PTPases AS POSITIVE MEDIATORS OF CELLULAR SIGNALING RESPONSES

Although these and other examples point to a role for PTPases in suppressing tyrosine phosphorylation-dependent signaling pathways, it seems clear that the PTPases cannot simply be regarded as antagonists of the PTKs. Several PTPases have now been shown to act positively in mediating signaling responses. In *Drosophila*, a mutation termed *corkscrew* causes abnormal development of the terminal head and tail structures during embryogenesis. This developmental pathway is believed to involve a cascade of phosphorylation events triggered by the activation of a transmembrane PTK termed *torso*. Cloning of the *corkscrew* gene revealed that it encodes an SH2 domain-containing PTPase more closely related to the ubiquitously expressed mammalian enzyme (which is termed Syp, SHPTP2, or PTP1D) than the hematopoietic cell-specific HCP molecule that underlies the murine *motheaten* phenotype discussed above (Perkins et al., 1992). Through a variety of genetic approaches it has been demonstrated that *corkscrew* functions positively in conjunction with a Ser/Thr kinase, D-*raf*, in transducing the *torso* signal. Similarly Syp, the mammalian homolog of *corkscrew*, is thought to play a role in potentiating the signal from growth factor receptor PTKs. Thus, at present, it appears that two closely related SH2 domain-containing PTPases possess a similar organization of structural motifs, yet serve very different physiological functions, one promoting the other antagonizing PTK-induced signaling pathways.

One of the best characterized examples of a positive role for a PTPase in signal transduction involves CD45, the prototype receptor PTPase. CD45 represents a family of proteins expressed exclusively on nucleated hematopoietic cells and which can occupy up to 10% of the surface of a lymphocyte. Multiple isoforms of CD45 have been identified that differ due to the variable expression of three exons encoding sequences at the extreme N-terminus of the protein. This leads to differences in protein sequence and patterns of glycosylation. Different CD45 isoforms are expressed in a highly regulated, cell type-specific fashion. In addition, individual T-cells can express more than one isoform in a pattern that varies with cell activation. These observations imply an important role for this PTPase in lymphocyte function, but only recently has the mechanistic basis of that function become apparent. A breakthrough came with the generation of T-cells that failed

to express CD45 (reviewed in Woodford-Thomas and Thomas, 1993). It was shown that CD45⁻ T-cell clones failed to proliferate in response to antigen or to cross-linking of CD3, but did respond normally to IL-2. Subsequently, a number of CD45-deficient T-cell lines were developed that were shown to be defective in T-cell receptor-induced signaling responses. Thus, unlike the normal parental lines, these CD45⁻ cells did not respond to T-cell receptor stimulation with the release of $Ca^{2+}$ from intracellular stores, or with the turnover of phosphatidylinositol that leads to the production of lipid-derived second messengers. Perhaps most striking was the observation that in CD45⁻ Jurkat cells there was no T-cell receptor-induced increase in cellular phosphotyrosine. These signaling defects could be rescued by restoring expression of the PTPase by transfecting back CD45 cDNA. Thus CD45 is required for coupling stimulation of the T-cell receptor to activation of these signaling pathways, indicating that CD45 plays a positive role in mediating signal transduction in response to T-cell activation. A similar situation is now known to be true of B-cells. These data present something of a paradox: ablation of expression of a PTPase results in the failure of T-cell receptor stimulation to elicit an enhancement in the level of phosphotyrosine. The resolution of this paradox has come from the observation that the Src- family kinases, $p56^{lck}$ (found in association with the T-cell surface accessory proteins CD4 or CD8) and $p59^{fyn}$ (found in association with the CD3 complex that interacts with the T-cell receptor) are abnormally regulated in CD45⁻ cells. Members of the Src family of PTKs have two major sites of tyrosine phosphorylation. There is a site of autophosphorylation in the catalytic domain and a site at the C-terminus, equivalent to Tyr 527 in Src itself, that when phosphorylated exerts an inhibitory effect on kinase activity. In CD45⁻ cells, these kinases accumulate in a form in which the C-terminal inhibitory site is hyperphosphorylated, and thus activity is repressed, although the relative importance of $p56^{lck}$ and $p59^{fyn}$ to a particular pathway varies with cell type. Therefore the signaling defect in CD45⁻ cells appears to be a failure to activate Src family kinases efficiently. Thus, under normal conditions, CD45, a PTPase, can actually promote tyrosine phosphorylation through the dephosphorylation and activation of a Src-family PTK (Figure 5). Such a concept reinforces the importance of intracellular compartmentalization in the control of PTPase activity to prevent inappropriate dephosphorylation of the downstream targets of the activated Src-family kinase.

A similar interaction has been observed between Src itself and the receptor-like PTPase PTPα, and implies a role for this PTK/PTPase couple in neuronal differentiation (see Pallen, 1993). PTPα is characterized by a small, highly glycosylated extracellular segment of 123 amino acid residues. It is expressed ubiquitously and its levels are enhanced during neuronal differentiation of two embryonal carcinoma (EC) cell lines and in neuroblastoma cells, with maximal expression preceding the morphological change to the neuronal phenotype. Overexpression of PTPα in P19 EC cells led to a change in the normal pattern of retinoic acid-induced differentiation *in vitro*. The wild type cells normally differentiate into endodermal or mesoder-

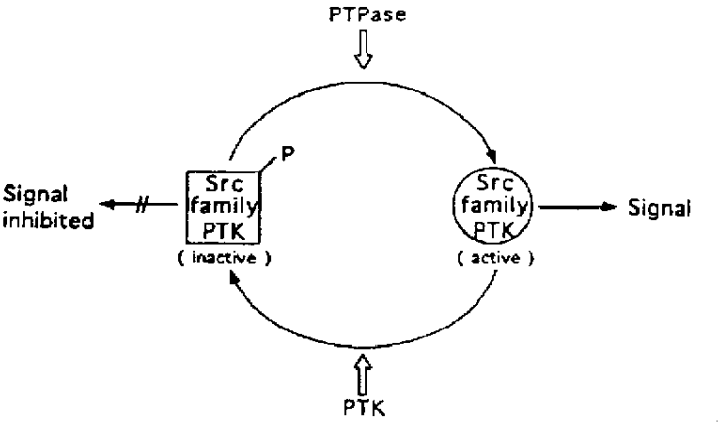


*Figure 5.* PTPases may act positively to promote signaling events *in vivo*. One mechanism by which PTPases may promote signaling responses is through the dephosphorylation and activation of members of the Src family of PTKs. Src family kinases have an inhibitory site of phosphorylation in their C-termini dephosphorylation of which by a PTPase promotes kinase activity and triggers the signaling function. Therefore by this mechanism a PTPase may actually promote tyrosine phosphorylation. Such a system requires strict subcellular compartmentalization to prevent the PTPase from also acting on the downstream targets of the Src family PTK. Reproduced from Sun and Tonks (1994).

mal cells; however, PTPα-overexpressors display a neuronal phenotype following retinoic acid treatment. These effects of PTPα coincide with the dephosphorylation of Tyr 527 in Src and activation of its kinase function (den Hertog et al., 1993). Similarly, overexpression of PTPα in rat embryo fibroblasts results in the dephosphorylation and activation of Src, and is accompanied by the generation of a transformed morphology.

The possibility that PTPases may antagonize the effects of oncogenic PTKs and may represent the products of tumor suppressor genes seems intuitively obvious. However, current evidence that increasingly points towards positive roles for certain PTPases in promoting cellular signaling responses raises the possibility that such PTPases may actually be the products of oncogenes. In the future their activation as a result of gene amplification or translocation events may be recognized as underlying certain proliferative disease states.

## THE DUAL SPECIFICITY PHOSPHATASES

The PTPases that I have described thus far share a common homology domain of ~240 residues that contains the unique signature motif that bears the active site cysteinyl residue and defines this family of enzymes. These PTPases are absolutely

specific for phosphotyrosyl residues in proteins and, at least *in vitro*, are somewhat promiscuous in their recognition of PTyr-containing substrates. Recently, a large sub-family of dual specificity phosphatases has been uncovered of which VH1 from *Vaccinia* virus was the first to be identified (Guan et al., 1991). Further examples have been detected in several pox viruses, yeast, mammalian cells, and even in a prokaryote, the cyanobacterium *Nostoc commune* (reviewed in Guan and Dixon, 1993). These enzymes differ from the classical PTPases in that although they contain the [I/V]HCXAGXXR[S/T]G signature motif they display little other structural similarity with this enzyme family. Furthermore, in many cases, they have been demonstrated to dephosphorylate both Tyr and Ser/Thr residues in proteins. Perhaps most strikingly these enzymes tend to display a very restricted substrate specificity *in vivo*. At least two of these enzymes have been shown to catalyze dephosphorylation events that are of fundamental importance to the control of cell function.

Tyrosine phosphorylation plays a crucial role in controlling the onset of mitosis in eukaryotic cells (see Atherton-Fessler et al., 1993 for review). The key cell cycle regulator $p34^{cdc2}$, which is a protein Ser/Thr kinase, is phosphorylated on adjacent residues, Thr 14 and Tyr 15, in the GXGXXG motif of the nucleotide binding loop. This phosphorylated form of $p34^{cdc2}$, which accumulates during interphase, is inactive. Dephosphorylation of Thr 14 and Tyr 15 in $p34^{cdc2}$ is a key step in activating this protein kinase and driving the transition into mitosis. Both of these dephosphorylation events are catalyzed by a single dual specificity phosphatase, $p80^{cdc25}$, and this mechanism of initiation of mitosis is highly conserved across all eukaryotic species. As observed for the classical PTPases, phosphorylation of Ser/Thr residues in its noncatalytic N-terminal segment and targeting to defined intracellular locations, as well as changes in its mRNA and protein levels, have all been implicated in the control of $p80^{cdc25}$ function in various cell systems.

Recent attention has focused on a widely-expressed dual specificity phosphatase that is the product of a murine immediate early gene, 3CH134. Both the mRNA transcript and the protein derived from this immediate early gene are induced rapidly and transiently following growth factor stimulation of quiescent cells. In addition, in human fibroblasts, this phosphatase has been recognized as the product

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***Figure 6.*** Signaling pathways involving MAP kinase. A common feature of the signaling pathways induced by diverse mitogenic stimuli is the activation of MAP kinases. The molecular details of the steps leading to MAP kinase activation are currently being elucidated. This figure summarizes the current appreciation of the cellular responses to activation of a growth factor receptor PTK by binding of its cognate ligand. Autophosphorylation of the receptor creates docking sites for the assembly of multiprotein complexes. These complexes include the receptor itself, adaptor proteins, such as GRB2, and nucleotide exchange factors, such as SOS, which trigger the accumulation of the active, GTP-bound form of Ras. (continued)

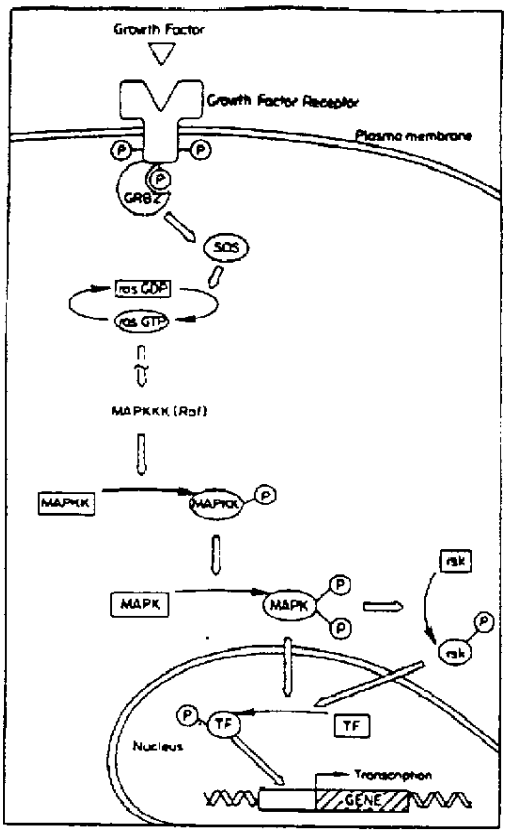


***Figure 6.*** (continued) The Ser/Thr kinase Raf is activated in response to accumulation of Ras-GTP. Although Ras and Raf physically interact this association is not sufficient for activation of Raf and the molecular intricacies of this activation event remain to be elucidated. Raf phosphorylates and activates MAP kinase kinase, which in turn phosphorylates both Tyr and Thr regulatory sites in MAP kinase with concomitant activation. Active MAP kinase is thought to promote transcription of genes required for the growth response either directly, by phosphorylation of transcription factors, or indirectly, through the phosphorylation and activation of another Ser/Thr kinase, $p90^{rsk}$, which may in turn phosphorylate transcription factors.

of a stress response gene, the expression of which is induced by stimuli such as heat shock. Complementary DNA has been cloned and the enzyme expressed and characterized. It possesses intrinsic phosphatase activity that is highly specific for MAP kinase *in vitro*. The MAP kinases have been implicated as common components of signaling pathways induced by diverse mitogenic stimuli. For example, growth factor-induced autophosphorylation of receptor PTKs creates specific binding sites for proteins possessing SH2 domains (reviewed in Pawson and Schlessinger, 1993) (Figure 6). One such class of proteins contains both SH2 and SH3 domains which serve as adaptors to link stimulation of a PTK to activation of Ras. Thus the adaptor protein, GRB2, binds to a particular site of autophosphorylation in the receptor PTK through its SH2 domain, and to a nucleotide exchange protein, SOS, through its SH3 domains. The SH3 domains in GRB2 recognize specific proline-rich motifs in SOS. Assembly of this multiprotein complex in the membrane triggers an SOS-induced conversion of Ras from an inactive GDP-bound form to an active GTP-bound state. Activated Ras then initiates a cascade of sequential phosphorylation events in which the Ser/Thr kinase Raf phosphorylates and activates MAP kinase kinase (also known as MEK) which is a dual specificity kinase that in turn phosphorylates both Thr 183 and Tyr 185 in MAP kinase. Phosphorylation of both Tyr and Thr regulatory sites is essential for MAP kinase activation. Once activated, MAP kinase can phosphorylate a number of substrates, including transcription factors, that are essential for triggering the expression of genes that are required for the mitogenic response. One such gene is 3CH134 that encodes the dual specificity phosphatase. Data have now been presented to show that this enzyme not only dephosphorylates MAP kinase *in vitro* but also dephosphorylates both the Tyr and Thr regulatory sites and inactivates MAP kinase *in vivo* (Sun et al., 1993). Furthermore, this enzyme appears to be absolutely specific for MAP kinase and is now called MKP-1 (MAP kinase phosphatase) to reflect this restricted specificity. Therefore, this phosphatase may feed back on the mitogenic signaling pathway by dephosphorylating and inactivating MAP kinase, and thus attenuate the signaling response and prevent uncontrolled growth and proliferation. In addition, the high degree of specificity shown by MKP-1 suggests that it may prove to be a powerful tool for defining physiological roles for MAP kinase.

## SUMMARY AND PERSPECTIVES

This is an exciting time to be working on the protein tyrosine phosphatase family of enzymes. Some 50 members of this family have been identified to date and although important progress remains to be made in identifying novel PTPases and determining their primary structure, we are now also able to move on towards confronting the challenge of elucidating the biological function of these enzymes. The picture has changed from the initial view that PTPases would merely serve as passive antagonists of PTK function. We now know that the PTPases will rival the

kinases both in their structural diversity and complexity, and in the sophistication of their regulation. Perhaps most importantly, the PTPases also have the potential to play an active role in promoting signaling events. Clearly continued study of this family should provide important new insights into the physiological importance of tyrosine phosphorylation.

## ACKNOWLEDGMENTS

Although I have cited some original papers in this article, due to space restraints I have largely referred to reviews to provide the reader with a source of additional information. I apologize to those authors whose primary papers are not included. Work in my lab is supported by grants from the National Institutes of Health/National Cancer Institute (CA53840 and CA64593), from The Council for Tobacco Research and from the Mellon Family, Hansen Memorial and Lauri Strauss Leukemia Foundations. I am also supported by a Pew Scholarship in the Biomedical Sciences. I thank Carol Marcinczuk for typing the manuscript.

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