Protein Tyrosine Phosphatases: A Diverse Family of Intracellular and Transmembrane Enzymes

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Protein tyrosine phosphatases (PTPs) represent a diverse family of enzymes that exist as integral membrane and nonreceptor forms. The PTPs, with specific activities in vitro 10 to 1000 times greater than those of the protein tyrosine kinases would be expected to effectively control the amount of phosphotyrosine in the cell. They dephosphorylate tyrosyl residues in vivo and take part in signal transduction and cell cycle regulation. Most of the transmembrane forms, such as the leukocyte common antigen (CD45), contain two conserved intracellular catalytic domains; but their external segments are highly variable. The structural features of the transmembrane forms suggest that these receptor-linked PTPs are capable of transducing external signals; however, the ligands remain unidentified. A hypothesis is proposed explaining how phosphatases might act synergistically with the kinases to elicit a full physiological response, without regard to the state of phosphorylation of the target proteins.

ONE OF THE MAIN LESSONS WE HAVE LEARNED OVER THE last 30 years regarding the control of cellular processes is that, of the two general mechanisms by which metabolic pathways can be regulated, that is, by allosteric control and covalent modification by protein phosphorylation, covalent regulation responds mainly to extracellular signals. Some ligands, which include hormones, growth factors or neurotransmitters, interact with their specific receptors to cause the release or synthesis of second messengers in reactions regulated by G proteins. Others increase the protein tyrosine kinase (PTK) activity of their receptors. In turn, these signals trigger a cascade of reactions allowing both the amplification of the original stimulus and the coordinate regulation of separate cellular processes through the pleiotropic action of the enzymes involved.

Protein tyrosine phosphorylation is implicated in the control of normal and neoplastic cell growth and proliferation (*1*). In this article, the structure and function of the family of specific protein tyrosine phosphatases (PTPs) and their interplay with the PTKs are described. It will not cover the serine-threonine phosphatases, which were discussed in recent reviews (*2*).

Properties of the Nonreceptor PTPs

PTP activity was first observed in membranes from A431 cells and in cells transformed with temperature-sensitive mutants of Rous sarcoma virus (*3, 4*). The partial purification and characterization of multiple forms of PTPs from various tissues and cell lines has been presented (*5–7*). PTP1B was isolated in homogeneous form from the soluble and particulate fractions of human placenta (*8*). The enzyme is active as a monomeric catalytic subunit of 37 kD. Although entirely specific for phosphotyrosine, the enzyme is not selective among substrate proteins bearing the phosphorylated residue. The specific activities in vitro of PTP1B for four artificial substrates are up to 1000 times higher than those of the retroviral or receptor-linked PTKs, and K_m's of the enzyme are in the submicromolar range. Thus, the potent activity of the PTPs may counteract the tyrosine kinases that promote uncontrolled growth when they are deregulated. On the other hand, the PTPs must also be tightly regulated to allow for those signals that are necessary for normal cell growth and development (*9*).

In addition to the classical inhibitors orthovanadate (*6*), molybdate (*6*), and zinc (*5*), PTP1B is inhibited by acidic polymers such as heparin or poly(Glu-Tyr) (*8*). The inhibition is noncompetitive, as if the phosphatase contained a high affinity inhibitory site separate from the catalytic center (*8*). Physiological counterparts for these anionic species have not been identified, but two heat-stable inhibitors from bovine brain have been described (*10*).

Structural Features of the PTP Gene Family

Sequence analysis of the human placenta PTP1B (*11*) demonstrated that it is comprised of a single N^{α}-acetylated polypeptide chain of 321 residues. Placental PTP1B is not structurally related to the serine-threonine–specific phosphatases (*11*), indicating that the phosphatases have evolved as separate families in contrast to the protein kinases, which all appear to be derived from a common progenitor (*12*). However, PTP1B was found to be homologous to each of two tandem domains in the cytoplasmic portion of the leukocyte common antigen (CD45) (*13*). CD45, a transmembrane protein, was shown to possess intrinsic tyrosine phosphatase activity (*14*). Subsequently, other transmembrane PTPs have been identified, indicating that the PTPs represent a diverse family of enzymes with both intracellular and integral membrane forms. The structural organization of CD45 and its transmembrane homologs resemble that of several integral membrane tyrosine kinases such as the epidermal growth factor receptor. Thus, these receptor-linked PTPs may be capable of initiating transmembrane signaling in response to external ligands.

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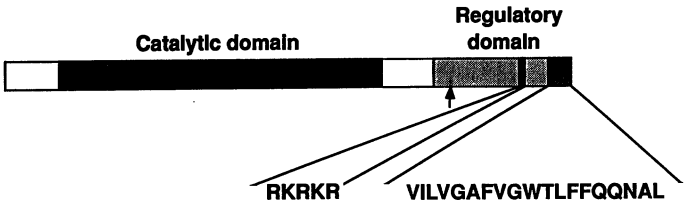


Fig. 1. Schematic diagram illustrating the structural organization of T cell PTP. The positions of the conserved catalytic domain and a putative regulatory domain are indicated. Sequences for a potential nuclear localization signal and hydrophobic COOH-terminal segment are given in single-letter codes as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. The arrow denotes the site where the corresponding cDNA was truncated to generate the 37-kD form of the enzyme.

Sequence analysis revealed a segment of approximately 230 residues conserved among two nonreceptor and four transmembrane PTPs (*11*). This segment was later identified in all PTPs, indicating that it comprises the catalytic domain. These domains have a common cysteinyl residue that is required for activity (*15–18*). These findings account for the known sensitivity of PTP1B to thiol-directed reagents (*8*) and suggests that this cysteinyl residue participates in the catalytic mechanism. This essential cysteine is located within a highly conserved segment of 11 residues with the consensus sequence, [I/V]HCXAGXXR[S/T]G, which is a hallmark of the PTP domain (see legend to Fig. 1 for abbreviations; X signifies an unconserved amino acid).

Isolation of cDNAs for the nonreceptor PTPs from human T cell (*19*), rat brain (*20*), and human placenta libraries (*21, 22*) has been reported. The latter two cDNAs encode PTP1B, but the T cell gene product represents a distinct enzyme displaying 65% sequence identity with PTP1B (74% within the catalytic domain) (*19*). In all cases, the cDNAs encode proteins with an extension of about 11 kD at their COOH-termini relative to the enzyme purified from placenta, which is a truncated product of the PTP1B gene. It is not clear whether truncation is a physiologically relevant process or an artifact of isolation. The exon organization of the human PTP1B gene indicates that truncation cannot be ascribed to RNA splicing (*22*). The sequences of the COOH-terminal extensions of all three nonreceptor PTPs are related; however, the sequence similarity is far lower than that among the corresponding catalytic domains. The sequence identity between the rat and human PTP1B COOH-terminal extensions is 49%, whereas that among the T cell PTP and PTP1B enzymes is only 18%. The last 19 residues of the COOH-terminal extension are hydrophobic and may be responsible for localization of the full-length enzymes to the particulate fraction (*23*) (Fig. 1). It is not known whether post-translational modifications such as acylation function in membrane localization. The T cell phosphatase has a stretch of positive charges (Fig. 1) similar to those identified as nuclear localization signals in certain protooncogenes (*24*), but there is no evidence that the enzyme occurs within the nucleus.

A nonreceptor PTP is encoded by plasmids from bacteria of the genus *Yersinia* that are responsible for a number of diseases including bubonic plague. Expression of this PTP gene is required for pathogenecity (*17*). In contrast to its mammalian counterparts, the *Yersinia* PTP has an unrelated, noncatalytic segment located upstream from the catalytic domain. Vaccinia virus also encodes a protein distantly related to the eukaryotic PTPs. This small 20-kD PTP retains the essential cysteinyl residue that characterizes the PTP family. Surprisingly, when expressed in bacteria, it dephosphorylates both phosphotyrosine and phosphoserine containing substrates (*18*). A cDNA encoding a 62-kD protein homologous to the PTPs was identified in *Schizosaccharomyces pombe*; like the Yersinia PTP, it has a noncatalytic segment of about 280 residues at the NH_2-terminus (*25*).

At least seven distinct transmembrane PTPs have been identified (Fig. 2 and Table 1), although some have not been isolated or

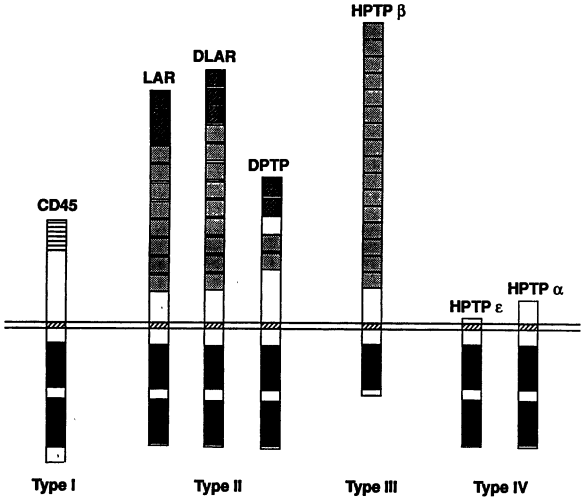


Fig. 2. Structural organization of transmembrane PTPs. Conserved catalytic domains are shown as solid segments. The region of CD45 encoded by differentially expressed exons is indicated by a segment possessing horizontal bars. Shaded boxes indicate the position of type III, fibronectin-like domains whereas crosshatched boxes designate immunoglobulin-like domains.

Table 1. PTP Isoforms. R, receptor; NR, nonreceptor.

PTP	Organism	Type	Size*	PTPase domains	Reference
CD45	Human	R type I	1120–1281	2	(*74*)
	Rat	R type I		2	(*75*)
	Mouse	R type I		2	(*76*)
LAR	Human	R type II	1881	2	(*77*)
DLAR	*Drosophila*	R type II	1997	2	(*15*)
DPTP	*Drosophila*	R type II	1439	2	(*15*)
HPTPβ	Human	R type III	1975	1	(*26*)
RPTPα	Mouse	R type IV	793	2	(*78*)
HPTPα	Human	R type IV	774	2	(*26, 32*)
HLRP	Human	R type IV	793	2	(*79*)
LRP	Mouse	R type IV	793	2	(*31*)
HPTPε	Human	R type IV	681	2	(*26*)
HPTPδ	Human	R type II?	>1523	2	(*26*)
HPTPγ	Human	R type ?	>610	2	(*26*)
RPTPγ	Human	R type ?	>535	2	(*32*)
HPTPζ	Human	R type ?	>613	2	(*26*)
RPTPβ	Human	R type ?	>530	2	(*32*)
PTP 1B	Human	NR PTP	435	1	(*21, 22*)
PTP 1	Rat	NR PTP	432	1	(*20*)
TC-PTP	Human	NR PTP	415	1	(*19*)
Yop 2b	*Yersinia*	NR PTP	468	1	(*17*)
VH1	Vaccinia virus	NR PTP	171	1	(*18*)
pyp^{1+}	*S. pombe*	NR PTP	550	1	(*25*)

*Number of amino acid residues.

expressed to show directly that they are active. With the exception of one isoform, HPTPβ (*26*), the intracellular segments of all transmembrane PTPs are remarkably similar, consisting of two catalytic domains. These domains are separated by a 58-residue segment with reduced sequence similarity. The two-domain motif is located about 78 to 95 residues from the transmembrane segment and is followed by a relatively short COOH-terminal sequence (Fig. 2).

As yet, no satisfactory nomenclature for the transmembrane PTPs has been devised. All but one of them have the same tandem catalytic domains displayed by CD45; therefore, to call some of these "leukocyte common antigen related" (or LAR) seems inappropriate. In contrast, they show a striking diversity in their external segments, and it is on these structural features that it would be more appropriate to base a classification scheme. While we are not suggesting a new nomenclature at this point, four PTP types can be distinguished: (type I) CD45 has a heavily glycosylated external segment and a conserved cysteine-rich region; (type II) LAR and the *Drosophila* enzymes DLAR and DPTP resemble neural cell adhesion molecules (NCAM's), L1, and fasciclin II in that they have two to three tandem immunoglobulin G–like domains linked to two to nine fibronectin type III (FNIII) repeats; (type III) the human HPTPβ and possibly HPTPδ contain only multiple-FNIII domains; and (type IV) the human enzymes receptor-PTPα (RPTPα) and HPTPε have much smaller external domains (123 and 27 residues, respectively). RPTPα may be glycosylated at eight sites.

The type I enzyme, CD45, may be unlike any other phosphatase because it is detected only on cells of hematopoietic lineage. Type II enzymes resemble molecules that are involved in homophilic cell-cell interactions (interactions between the same surface molecule on opposing membranes), raising the possibility that they too may modulate development and morphogenesis (*27*). The external segments of type III molecules may share some of the cell-adhesion properties of fibronectin and mediate heterophilic interactions (those between two different molecules) with ligands located on other cells or within the extracellular matrix (*28*). Some of the type IV receptors (such as RPTPα) are broadly distributed and may have a more general function in signal transduction. It is unlikely that the very short external segment of HPTPε serves as a receptor for circulating factors, but it may be a ligand for other receptors.

Eleven distinct PTP isotypes have been identified in mammals (Table 1). In the protochordate *Styela plicata* (sea squirt), 27 distinct PTPs have been detected by polymerase chain reaction (PCR) techniques (*29*); it remains to be established whether these represent novel enzymes or species-specific isoforms of known PTPs. The large number of isoforms within a single invertebrate organism indicates that the PTP gene family may be similar in complexity to that of the PTKs (*12*). Alternative mRNA splicing may also contribute to the diversity of PTP isoforms. Six isoforms of CD45 result from the differential usage of three exons encoding sequences in the extracellular segment (*30*). Alternative splicing within the first catalytic domain of RPTPα has been described (*31*) and may also occur within its extracellular segment (*32*).

CD45: A Receptor-Linked PTP

CD45 is an abundant transmembrane glycoprotein (comprising up to 10% of the cell surface) found only in hematopoietic cells. It consists of an external segment with the hallmarks of a ligand binding motif, a single transmembrane stretch, and an intracellular segment that contains two tandem PTP-like domains (*30*). An unusual sphingolipid containing myristate appears to be covalently attached to the extracellular segment (*33*). The various isoforms resulting from alternative splicing are differentially expressed on leukocyte subsets and are glycosylated to different extents, which might alter their ligand binding properties. CD45 has intrinsic PTP activity (*14*), but, unlike PTP1B, it preferentially dephosphorylates myelin basic protein (MBP) rather than reduced, carboxamidomethylated, and maleylated lysozyme (RCML) (*34*).

In mutant T cell lines that do not express either CD45 protein or mRNA (CD45), stimulation of the T cell receptor (TCR) failed to elicit phosphatidylinositol (PI) turnover (*35*). Transfection with cDNA for CD45 restored induction of PI turnover, indicating that CD45 is essential in linking the TCR complex to PI metabolism, presumably through at least one dephosphorylation event.

In these $CD45^-$ cells, the total extent of tyrosine phosphorylation in response to TCR stimulation was greatly reduced (*36*). Although the identity of the kinase(s) involved has not been established, the products of the *lck* and *fyn* genes have been suggested as likely candidates. The tyrosine kinase $p56^{lck}$ is associated with the internal domains of CD4 and CD8 (*37*); one of its major substrates is thought to be the zeta chain of the TCR-CD3 complex. In contrast $p59^{fyn}$ interacts with the TCR (*38*). Like other members of the Src family, both of these kinases are inhibited by phosphorylation of tyrosyl residues located near their COOH-termini (*39*); hence, their activity can be controlled by dephosphorylation. The kinase $p56^{lck}$ may be a substrate for CD45. In isolated lymphocyte membranes, CD45 is responsible for a two- to threefold activation of $p56^{lck}$ which has been correlated with the dephosphorylation of Tyr^{505} (*40*). Increased phosphorylation of Tyr^{505} in $CD45^-$ mutant T cell lines (*41*) suggests that CD45 may dephosphorylate $p56^{lck}$ in vivo. Antibody-induced cross-linking of CD4 and CD45 also results in dephosphorylation of $p56^{lck}$, which may account for the enhanced release of intracellular Ca^{2+} observed under similar conditions (*42*). In B cells, CD45 has been suggested to be a component of the antigen receptor complex and to have a critical role in antigen-induced activation (*43*).

Although most of the other transmembrane PTPs also appear to be equipped to function in primary signal transduction events, their physiological roles remain unidentified. As mentioned above, the prevalence of forms with extracellular domains resembling fibronectin or NCAMs raises the possibility that they may take part in cell adhesion, thereby controlling growth and differentiation in response to cell-cell or cell-matrix interactions. Obviously, by opposing the effects of the PTKs, these enzymes could participate in processes such as contact inhibition.

Regulation and Potential Cellular Functions of the Nonreceptor PTPs

When expressed in baby hamster kidney (BHK) cells, the full-length 48-kD form of the T cell PTP is recovered as a complex of >650 kD in the particulate fraction (*23*). With RCML as substrate, it displays low activity that is increased 10 to 20 times by tryptic cleavage of the 11-kD COOH-terminal tail. Expression of a 37-kD form with the COOH-terminal tail deleted yielded a product that was predominantly soluble and fully active without trypsin treatment (*23*). These data suggest that the enzyme contains a COOH-terminal segment that is critical for localization and regulation of the enzyme (Fig. 1). The NH_2-terminal noncatalytic domain of the *Yersinia* PTP (*17*) may have a similar function.

As with the tyrosine kinases, identification of physiological substrates of PTPs has been difficult though several observations suggest potential candidates. Microinjection of PTP1B into *Xenopus* oocytes retarded insulin-induced maturation and abolished the activation of a kinase phosphorylating a peptide derived from the ribosomal protein S6, an effect which coincided with the dephosphorylation of tyrosyl residues within several proteins, including one presumed to be the β subunit of the insulin or insulin-like growth factor I receptor (*44*). Overexpression

of the full-length enzyme in BHK cells rendered actin filaments resistant to cytochalasin-induced disassembly (*45*), suggesting that the PTPs might act on the complex membrane-associated structures where actin bundles terminate. These structures include the proteins talin, vinculin, paxillin, ezrin, α-actinin, and others that are concentrated at regions of cell-substratum contact or cell-cell interaction (*28, 46*). Talin and vinculin are phosphorylated on tyrosyl residues and are thought to be associated with the cytoplasmic domain of integrin receptors, which also contain phosphotyrosine. Actin fibers are also anchored to the band 4.1-spectrin-ankyrin system underlying the erythrocyte plasma membrane (*47*). Fodrin, a homolog of brain spectrin, was reported to interact with CD45 (*48*). Other cytoskeletal proteins that are phosphorylated or Ser-Thr residues, such as vimentin, which is phosphorylated by $p34^{cdc2}$ (*49*), or caldesmon, which is a substrate for the Ca^{2+}/calmodulin–dependent protein kinase II (*50*) could be indirectly affected. Such sites of action of the PTPs would be consistent with the localization of the enzymes in the particulate fraction.

Possible Involvement of PTPs in Control of the Cell Cycle

In contrast to the full-length enzyme, expression of the truncated 37-kD form in BHK cells produced striking changes in morphology in some cases; the cells became multinucleate, apparently due to a defect in cytokinesis (*51*). Even more unexpected was the fact that nuclear division was asynchronous. While cases of multinucleation are well documented, to our knowledge, nuclear division is always synchronous. The truncated PTP therefore seems to interfere with a synchronization signal.

One candidate substrate for PTPs is $p34^{cdc2}$, a protein serine-threonine kinase that functions in a cell cycle control mechanism ubiquitous in eukaryotes (*52*). The activity of $p34^{cdc2}$ varies dramatically during the cell cycle, a phenomenon associated with changes in its phosphorylation state and alterations in its association with various regulatory molecules (*53*). Phosphotyrosine is first detected in $p34^{cdc2}$ at the onset of DNA synthesis and rises to a maximum in G_2 phase. Activation of $p34^{cdc2}$ in *S. pombe* requires dephosphorylation of a tyrosyl residue (*54*) in the adenosine triphosphate (ATP)–binding site (and an adjacent threonine in higher eukaryotes). This activation drives the cell from G_2 into M phase.

The PTPs that act on $p34^{cdc2}$ have not yet been unequivocally identified. However, $p80^{cdc25}$ has been associated with the dephosphorylation of $p34^{cdc2}$ (*54*). A distant structural relation between $p80^{cdc25}$ and a known PTP, VH1 from vaccinia virus, has been noted (*55*); significantly, VH1 is the only PTP showing specificity for both serine-threonine and tyrosine. The T cell PTP, which is not restricted to lymphocytes (*56*), dephosphorylates and activates $p34^{cdc2}$ from *S. pombe* in vitro and in vivo (*57*).

Interaction Between Kinases and Phosphatases

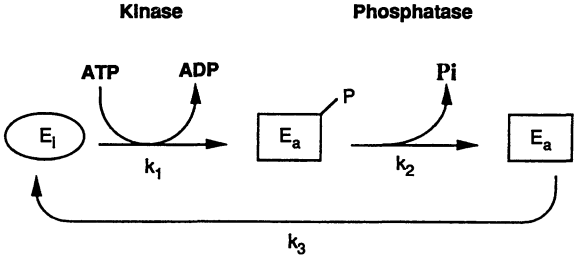
Originally, protein phosphatases were thought of as enzymes that served merely to counteract the action of the kinases, whether this resulted in the activation or inhibition of a target enzyme. A classical example is that of phosphorylase phosphatase, which causes the inactivation of phosphorylase and activation of glycogen synthase. This reciprocal effect was thought to prevent a futile cycle resulting in ineffectual utilization of ATP (*58*). However, it soon became apparent that these interconvertible enzymes do not exist in fully active or inactive forms; that is, the kinases and phosphatases do not simply serve as on-off switches. It was shown that the cyclic interconversion of regulatory enzymes represents a dynamic process in which the steady-state equilibrium between active and inactive forms varies gradually depending on many parameters determined by the metabolic state of the system (*59*). Nonetheless, it was accepted that the state of activity of an interconvertible enzyme would depend essentially on the ratio of its phosphorylated to nonphosphorylated forms. However, other mechanisms can be envisioned, particularly for the tyrosine kinases and phosphatases.

There are obvious distinctions between the systems controlled by tyrosine as opposed to that of serine-threonine phosphorylation: First, in many cases, tyrosine phosphorylation appears to be transient and substoichiometric; yet, normal physiological responses can be elicited. Second, proteins phosphorylated on tyrosyl residues are often phosphorylated on seryl and threonyl residues (*1, 52–54, 60–63*) with the implication that these two types of modification could act cooperatively with one another.

Because most protein tyrosine kinases are associated with the membrane, tyrosine phosphorylation has been regarded as a primary signal capable of affecting the state of activity of secondary enzymes, downstream (*61, 63*). In such a cascade, receptor kinases would serve a triggering role, relying on the longer-lived serine-threonine phosphorylation of the secondary enzymes to sustain the signal. Tyrosine phosphatases would guarantee that the activity of the tyrosine kinases would be transient, thus contributing to the desensitization process.

Phosphatases May Act Synergistically with Kinases

As described above, PTPs can cause a direct activation of $p56^{lck}$ and $p34^{cdc2}$. However, phosphatases might play a far more general role, contributing positively to the action of any kinase irrespective of whether they are repressed by phosphorylation. In the following hypothesis, an interconvertible enzyme can exist in inactive and active phosphorylated forms, E_i and E_a, respectively. We can assume that the enzyme will retain its active conformation for a finite period of time after undergoing dephosphorylation according to the following scheme:



If return of E_a to E_i were slow compared to the forward reactions, and $k_2 \gg k_3$, the state of activity of the system would depend essentially on the ratio of E_a to E_T (the total amount of enzyme) and be independent of the state of phosphorylation of E_a (*64*). Furthermore, if k_3 were rate-limiting, regulation would occur at this step, rather than during the kinase or the phosphatase reactions. This hypothesis describes a simple hysteretic system (*65*). One of its attractive features is that the change in conformation that occurs during the $E_a \rightarrow E_i$ transition could be controlled by interaction with metabolites or other components of the system, protein-protein interaction or by phosphorylation at a secondary site [for example, on seryl or threonyl residues (*66*)]. Such a scheme would readily account for the discrepancy observed between the level of tyrosine phosphorylation and a cellular response.

A similar hysteretic reaction was shown to occur during the

activation of a cytosolic form of type 1 serine-threonine phosphatase, which exists in an inactive complex with inhibitor-2 (I-2). Phosphorylation of I-2 activates the catalytic subunit. However, the active conformation is maintained after autodephosphorylation of I-2 since the return to the inactive state is slow (*67*). Here also, there is no correlation between the state of phosphorylation of the complex and its enzymatic activity. Furthermore, phosphorylation at secondary sites by casein kinase II (CKII) could stabilize the active conformation (*68*).

However, if the kinase were inhibited by the product of its reaction, E(P), the phosphatase could directly facilitate the formation of E_a; kinase inhibition would be relieved by the phosphatase and the cellular response would increase even though the level of substrate phosphorylation would decrease. Every phosphorylation-dephosphorylation cycle would generate the active conformation. Kinases and phosphatases would act in concert, serving as the "hand crank" that at every turn "winds up" the enzyme into its active state.

Prospects

While much progress has been made in the identification of PTP isoforms, little is known about their mechanism of action or regulation. It is not clear why so many isoforms exist, but the combination of catalytic domains with a variety of noncatalytic structures probably provides for distinct substrate specificities, modes of regulation, and localization.

Even though different forms of the PTPs have shown some substrate preferences [for example, between RCM-lysozyme and MBP (*34*)] no consensus recognition sequence has emerged. Negative charges, mostly upstream, often surround tyrosine kinase autophosphorylation sites. There is no compelling evidence for a "one kinase–one phosphatase" relationship for either the tyrosine-specific or the serine-threonine–specific enzymes. On the other hand, the different isozymes might display different sensitivities to effectors such as divalent cations, polyamines, negatively charged polymers or protein activators or inhibitors.

As described above, phosphorylation of tyrosyl residues together with seryl-threonyl residues has been implicated in the regulation of several protein kinases (*1, 52–54, 60–63*). The possibility that the PTPs are regulated similarly by protein phosphorylation is currently being addressed. The phosphorylation state of one or more residues in a protein may modulate the rate of phosphorylation of a distinct site in the same substrate by protein kinases. For example, glycogen synthase kinase-3 requires prior phosphorylation of its substrate by casein kinase-2. This type of regulation might apply to CREB (cAMP response element binding protein) (*66*); similarly PMA, presumably through activation of PKC, increases tyrosine as well as threonine phosphorylation of a 42-kD protein (*69*). For the PTPs, similar mechanisms may provide an additional tier of control at the level of the PTP substrate itself. In adipocytes, addition of okadaic acid (a potent inhibitor of the serine-threonine phosphatases, protein phosphatase 1 and protein phosphatase 2A) increases tyrosine phosphorylation of 42-kD and 38-kD proteins presumably myelin basic protein kinases (*62*). One of several possible explanations for this effect is that serine-threonine phosphorylation at remote sites renders these substrates resistant to the action of PTPs. Whether the ability of PTPs to act upon specific phosphotyrosyl residues is modulated by interplay with second sites of phosphorylation remains to be established but is certainly a possibility that merits further study.

The full-length and truncated forms of the T cell PTP are found in distinct subcellular fractions and display differential effects on cell division and actin assembly. These observations illustrate the importance of having PTP isoforms localized within distinct compartments where they can regulate specific cellular events. The diversity among isoforms probably reflects this requirement for localization. Although nothing is known regarding changes in PTP expression and localization during development and differentiation, it is likely that some isoforms take part in these processes.

The ligands that regulate transmembrane PTPs have not been identified. With PTP type II and III receptors, it seems unlikely that they are circulating factors; interaction with other surface antigens perhaps on other cells seems more likely. Several transmembrane PTPs, including CD45, display high basal activity in vitro; hence it is not clear whether ligand binding increases or suppresses their activity. Alternatively, ligands could cause the receptors to aggregate or come into contact with other cell surface antigens, or both, generating a positive or negative response depending upon the molecules with which the PTPs interact.

The functional significance of the two-domain structure of PTPs is unclear. It has been suggested that only the NH_2-terminal domain is active (*16*). However, one cannot conclude that the second domain is nonfunctional as it may have a different specificity and its appropriate substrates may not yet have been identified. It is also possible that activity of domain II may be specifically regulated by ligands. While tandem catalytic domains are rare [they have been reported for S6 kinase (*70*) and creatine kinase from sea urchin sperm flagella (*71*)], many regulatory enzymes have homodimeric structures that are important for their regulation. Similarly, the two PTP domains could be involved in cooperative interactions. In this regard, domain II of CD45 is particularly interesting since it has a 19-residue insert bearing a putative phosphorylation site for casein kinase II that could modulate either domain.

Aberrant phosphorylation of tyrosyl residues in proteins can lead to oncogenic transformation. This may arise through either the deregulation or overexpression of a PTK or the underexpression of a PTP. For instance, treatment of NRK cells with vanadate, one of the actions of which is to inhibit PTPase activity, increased the amount of phosphotyrosine in the cells and generated a transformed phenotype (*72*). Therefore the possibility that the PTPs may function as anti-oncogenes will continue to provide a focus of attention. Attempts are being made to correlate the chromosomal localization of PTP genes with lesions known to be associated with various neoplasias (*22, 73*). Receptor-linked PTPγ was shown to localize in a region of chromosome 3 that is frequently deleted in renal cell and lung carcinomas. A PTPγ allele was lost in approximately half the tumor samples examined, suggesting that it is a candidate tumor-suppressor gene (*73*). Conversely, overexpression of a PTP may suppress or even reverse cellular transformation, with the exciting prospect that the PTPs may serve as probes with which to define the identity of the substrates of tyrosine phosphorylation and their function in oncoprotein action. The large size of the PTP gene family may be indicative of the importance of these enzymes to the control of cell growth and signal transduction. As with the protein kinases, multiple PTPs may be required to fine-tune the highly integrated pathways that regulate eukaryotic cells.

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