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Structure, Regulation, and Function of Protein Tyrosine Phosphatases

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The phosphorylation of tyrosyl residues in proteins is an essential aspect of many signal transduction events, including the control of both normal and neoplastic cell growth and proliferation. Since the earliest observations of tyrosine phosphorylation, it has been appreciated that this is a reversible process in which the net level of phosphate in a target substrate reflects the balance between the competing action of kinases and phosphatases. Thus, in cells transformed by temperature-sensitive mutants of Rous sarcoma virus, an elevation in the levels of phosphotyrosine is observed at the permissive temperature, at which the kinase is active; however, if the cells are shifted to the nonpermissive temperature, at which the kinase is inactivated, a rapid dephosphorylation of tyrosyl residues ensues due to the action of protein tyrosine phosphatases (PTPases) (Sefton et al. 1980). The last 10 years has witnessed great progress in the characterization of the protein tyrosine kinases, whereas the PTPases, although equally important, remained relatively neglected until recently. It is now apparent that the PTPases constitute a large, structurally diverse family of both nontransmembrane and receptor-linked, transmembrane proteins. In this paper, we review briefly some of the recent progress in the characterization of the structure, function, and mode of regulation of the PTPases.

Structural Diversity of Members of the PTPase Family

An enzyme referred to as PTP1B was the first tyrosine phosphatase to be isolated in homogeneous form (Tonks et al. 1988a,b). Determination of its amino acid sequence revealed two striking observations (Charbonneau et al. 1988). First, it is not structurally related to the Ser/Thr-specific protein phosphatases. Thus it appears that, unlike the kinases which are all derived from a common ancestor, the phosphatases have evolved in separate families. Second, however, a striking homology was detected between PTP1B and each of the tandem repeated intracellular domains of the leukocyte common antigen, CD45. CD45 is a transmembrane protein expressed exclusively on hematopoietic cells (Thomas 1989). Its structure can be described in terms of an intracellular segment, comprising two PTPase-related domains, a single transmembrane sequence, and an extracellular segment that bears the hallmarks of a ligand-binding motif. Thus, the exciting possibility was raised that CD45 may be regarded as a prototype for a novel family of receptor-linked molecules with the potential to initiate signal transduction pathways via the ligand-modulated dephosphorylation of tyrosyl residues in proteins. This was strengthened by the subsequent confirmation that CD45 displays intrinsic PTPase activity (Tonks et al. 1988c). Following these observations, many different PTPase isoforms have been described in a wide variety of tissues and cell lines (Fig. 1).

More than ten distinct transmembrane PTPases have now been identified by various cloning strategies (Streuli et al. 1988, 1989; Kaplan et al. 1990; Kreuger et al. 1990; Matthews et al. 1990; Gebbink et al. 1991). With the exception of HPTPβ (Kreuger et al. 1990), which bears a single catalytic domain, the intracellular segments of the receptors are very similar, comprising two PTPase-related domains. The functional significance of such an arrangement remains unclear. Obviously, oligomeric enzymes have the inherent capacity for cooperative interactions between the catalytic centers; however, suggestions have been made that only the amino terminal of the two PTPase-related domains is active (Streuli et al. 1990). Interestingly, in domain II of receptor PTPases γ and ζ (according to Streuli et al. 1990), the conserved active site cysteine residue described below is replaced by aspartate, suggesting that at least in these enzymes, this domain may not be catalytic. Nevertheless, it remains a possibility that the second domain may have a distinct specificity from the first with its substrates yet to be identified, or even that it may be modulated specifically by ligands. Preliminary observations by at least one group indicate a low level of activity in the isolated second domain of RPTPα in vitro (Wang and Pallen 1991). Although the intracellular segments of the various receptor PTPases are closely related, they diverge in their extracellular segments, a feature that presumably indicates a similarly diverse array of ligands. Although CD45 may be regarded as a prototype receptor-linked PTPase, as expected, the other members of the family are not restricted in their expression to hematopoietic cells. LAR, which is expressed in a variety of tissues, displays structural similarity to the N-CAMs in its extracellular segment, comprising 3 immunoglobulin-like and 8 fibronectin type III-like domains. HPTPβ has the longest extracellular segment identified thus far, comprising 16 repeated fibronectin type III domains (1599 residues), whereas

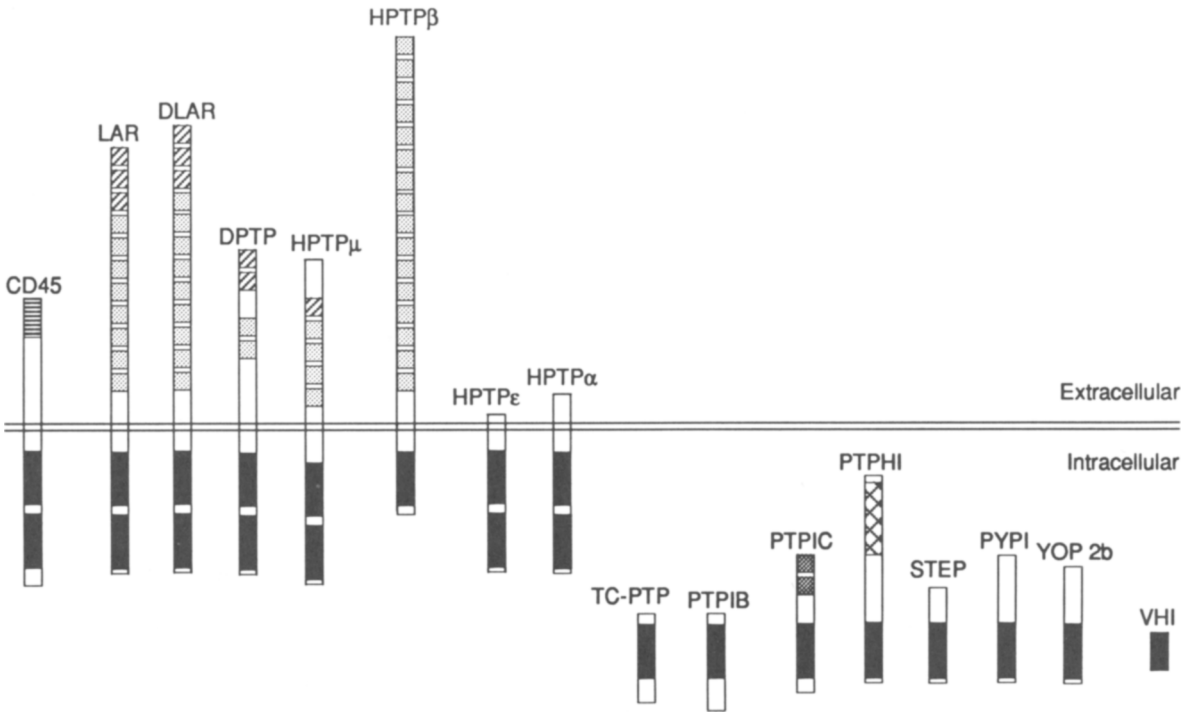

Figure 1. Structural organization of protein tyrosine phosphatases. Conserved catalytic domains are shown in black. The PTPases can be categorized as transmembrane, receptor-like, or non-transmembrane molecules. The receptor-like species can be subdivided into four types based on the structure of their 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 amino terminus (horizontal shading) are differentially expressed. Type II, containing immunoglobulin-like (diagonal lines) and fibronectin type III-like (stippled) domains, includes LAR, DLAR, DPTP, and HPTPμ. Type III bear multiple fibronectin type III repeats, such as in HPTPβ, which is also characterized by the presence of only one PTPase domain in its intracellular segment. Type IV, such as α and ϵ, have very small, glycosylated extracellular segments. Multiple non-transmembrane forms have also been identified. In TCPTP and PTB1B, and carboxy-terminal noncatalytic segment appears to play a role in modulating activity and controlling subcellular localization. Several PTPases bearing noncatalytic amino-terminal segments have now been identified. These segments include two SH2 domains in PTP1C, a band 4.1 homology domain in PTPH1, and structures apparently unrelated to sequences in the data base in STEP, PYP1, and YOP2b. The vaccinia protein VH1 is smaller than the other PTPase-related segments and presumably encodes only a catalytic domain. This is not an exhaustive list and the family continues to expand. A number of partial cDNAs for additional PTPases have also been identified but are not included.

the very short external domains of HPTPα and ϵ comprise only 123 and 27 residues, respectively. It is tempting to speculate that LAR may participate in adhesion reactions through homophilic interactions between the extracellular segments of these molecules on adjacent cells. Interestingly, the receptors for a number of soluble low-M_r factors have been shown to contain fibronectin type III "modules" (Norton et al. 1990; Patthy 1990). Although these structures may simply be indicative of segments important in protein:protein interaction, one could also speculate that the activity of HPTPβ may be influenced by soluble ligands or even members of the integrin family. At present, we still await data to suggest the identity of PTPase ligands.

In the initial flurry of activity concerning the identification of novel PTPases, the receptor-linked forms predominated. Now a diverse array of nontransmembrane PTPases have also been identified. The cDNA for a PTPase closely related to PTP1B was isolated originally from a human peripheral T-cell library (Cool et al. 1989). This molecule, TCPTP, is now known to display a broad tissue distribution. Comparison of these two sequences with those of CD45 and LAR revealed a conserved segment of approximately 230 residues, which most likely represents the catalytic domain and has been found in all the PTPases identified subsequently (Charbonneau et al. 1989). Within this conserved domain is a common cysteinyl residue that is essential for activity, explaining the sensitivity of these enzymes to thiol-directed reagents. The sequence surrounding this cysteine, [I/V]HCXAGXXR[S/T]G, is highly conserved, representing the signature motif that characterizes members of this family. Guan and Dixon (1990), on searching the NBRF data base, observed this motif in the Yop2b protein of the pathogenic bacterium *Yersinia* and the VH1 protein of the pox virus, vaccinia (Guan et al. 1991a). In both cases, the authors have demonstrated phosphatase activity intrinsic to the protein. In fact, for *Yersinia*, which is the causative agent of the plague or Black Death, the PTPase activity of the Yop protein is essential for virulence. In a murine macrophage cell line-model system, Yop2b pro-

motes the dephosphorylation of proteins of 120 kD and 60 kD, the identities of which remain to be established (Bliska et al. 1991).

The first nontransmembrane PTPases described, the low-M_r enzymes PTP1B and TCPTP, display a similar structural organization—the catalytic domain is situated in the amino-terminal portion of the molecule, and the carboxy-terminal segment serves a regulatory function (Cool et al. 1990). However, in many of the more recent additions to the family, the PTPase domain is carboxy-terminal, whereas the amino terminus is noncatalytic. Several groups have isolated cDNAs for a PTPase in which the large noncatalytic amino terminus contains two adjacent SH2 (Src homology region 2) domains (Shen et al. 1991). SH2 domains are found in several cytoplasmic proteins that serve a signaling function, including phospholipase-Cγ, GAP, PI3 kinase, and several protein tyrosine kinases, including src itself, and are involved in binding to tyrosine phosphorylated proteins (Koch et al. 1991). Whether they serve to target this PTPase to specific substrates or to modulate phosphatase activity remains to be established. In addition, a PTPase which in its amino-terminal segment displays identity to the cytoskeletal-associated proteins, band 4.1, ezrin, and talin, has been identified and is described in detail below (Yang and Tonks 1991). Furthermore, the Yop proteins from *Yersinia* (Guan and Dixon 1990), a molecule termed PYP1 from *Schizosaccharomyces pombe* (Ottilie et al. 1991), and STEP, isolated as a neural-specific cDNA from a rat striatal library (Lombroso et al. 1991), all contain amino-terminal segments that are not related to sequences in the data base and whose function remains to be established. An additional PTPase comprising essentially a catalytic domain alone has also been identified in *Saccharomyces cerevisiae* (Guan et al. 1991b).

At present, little is known about many of these PTPases except for their structure; in fact, for several, intrinsic activity remains to be established. The next goals will involve progressing beyond structure to characterize the activity, substrate specificity, mode of regulation, and, thus, physiological function of these enzymes.

Regulation of PTPase Activity

In vitro, when assayed with artificial substrates, PTP1B as isolated from human placenta displays a submicromolar K_m and a very high specific activity, some 1–3 orders of magnitude in excess of that of the PTKs (Tonks et al. 1988b). Similar levels of activity against an appropriate substrate were observed in preparations of CD45 (Tonks et al. 1990). These features suggest that the PTPases will effectively modulate the level of cellular phosphotyrosine. Furthermore, one would anticipate that the activity of the PTPases must be tightly regulated so as to permit the normal function of the PTKs. The mechanisms by which this is achieved are now beginning to come to light.

For the receptor PTPases there is the potential for control of activity through interaction with ligands. It remains unclear as to exactly what would be the effect of ligand binding. If the high basal activity of CD45 toward phosphotyrosyl myelin basic protein (MBP) is a general feature of receptor PTPases and is also observed with physiologically relevant phosphotyrosyl proteins within the cell, then it seems unlikely that the binding of ligands to the extracellular portion would enhance activity further. It is possible that in the basal condition only one of the two PTPase domains is active and the second becomes activated upon ligand interaction. Alternatively, the function of ligands may be to inhibit activity or perhaps even to control the localization of the PTPase on the surface of the cell, thus modulating the activity by restricting the spectrum of proteins with which it can interact.

The nontransmembrane enzyme, PTP1B, was purified from human placenta as a monomeric catalytic subunit of 37 kD (Tonks et al. 1988a). However, when cDNAs for this enzyme were isolated, they were observed to encode a molecule of approximately 50 kD that bore an extension of about 11 kD at the carboxyl terminus (Brown-Shimer et al. 1990; Chernoff et al. 1990; Guan et al. 1990). Similarly, the cDNA for a closely related molecule (74% identity in the catalytic domain), termed TCPTP, also encodes a 48-kD PTPase with a carboxy-terminal extension relative to the placenta enzyme (Cool et al. 1989). Thus, it now appears that PTP1B was isolated originally as a truncated species, although whether or not this truncation represents a physiologically relevant processing event remains to be established.

The properties of the 48-kD form of TCPTP were investigated following its expression in BHK cells (Cool et al. 1990). It was found to be associated with a particulate fraction isolated by low-speed centrifugation of a cell extract; solubilization of the PTPase required treatment with detergent. When the detergent lysate was subjected to gel filtration, the PTPase was recovered in a very high M_r complex, eluting essentially in the void volume of a Superose 12 column. Perhaps most importantly, when activity in the lysates was assayed with reduced carboxamidomethylated and maleylated (RCM) lysozyme, the expressed enzyme displayed little activity unless first subjected to limited trypsinization. This proteolytic activation was accompanied by the removal of the carboxy-terminal tail to generate a molecule of approximately 33 kD. When a truncated 37-kD form of the enzyme, in which the carboxy-terminal segment was deleted by insertion of a premature stop codon, was expressed, it was found to be no longer exclusively particulate, no longer associated with a high-M_r complex, and now spontaneously active toward RCM lysozyme. Similarly, when both 48-kD and 37-kD forms were expressed in Sf9 cells using the baculovirus system and purified to homogeneity, the truncated species displayed a very high activity toward RCM lysozyme, whereas the full-length form was relatively inactive unless first subjected to limited trypsinization (Zander et al. 1991). Interest-

ingly, the full-length enzyme was also activated by MBP and displayed a potent activity toward phosphotyrosyl MBP in the absence of trypsin. It would appear that the carboxy-terminal segment of TCPTP plays a role both in modulating the activity of the catalytic domain and in controlling the localization of the enzyme within the cell.

When BHK cells are stimulated with platelet-derived growth factor (PDGF), the phosphorylation of tyrosyl residues in several proteins is induced. When this pattern is compared to that induced by PDGF in BHK cells expressing either the 48-kD or 37-kD forms of TCPTP, both forms of the phosphatase lead to a reduction in the level of phosphotyrosine (Cool et al. 1990). Thus, there are mechanisms within the cell to permit the manifestation of the activity of the full-length TCPTP. Comparison of the maximal levels of PTPase activity detectable in extracts of BHK cells expressing either the 48-kD or 37-kD forms of TCPTP indicated that the cell can tolerate much higher levels of the full-length form of the enzyme than the truncated. In addition, expression of the 37-kD form induces significant aberrations in the cell cycle not seen with the full-length enzyme (D.E. Cool et al., in prep.). The 37-kD-expressors develop a multinucleated phenotype, apparently due to a defect in cytokinesis. Furthermore, in the resulting syncytial cells, the nuclei divide asynchronously. Within a single cell, one could detect nuclei in interphase together with nuclei in mitosis, suggesting that the phosphatase disrupted the signals which maintain nuclear synchronization. The nature of the substrates involved in these effects remains unclear; however, these data further attest to the importance of the carboxy-terminal segment of TCPTP in the capacity of the cell to modulate its activity.

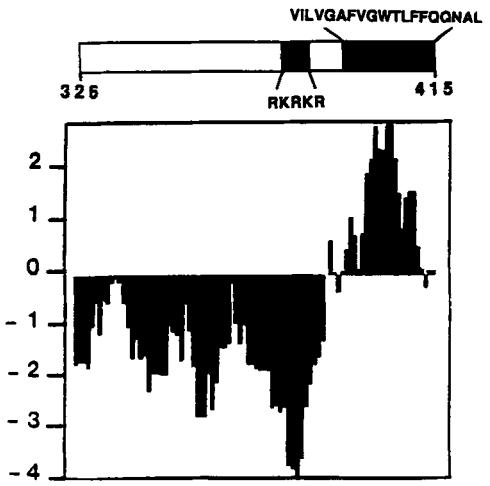

Figure 2. Structural features of the carboxy-terminal segment of TCPTP. The bar depicts the carboxy-terminal segment of TCPTP schematically, indicating the position of the putative nuclear localization signal (residues 377–381) as well as the position and sequence of the hydrophobic carboxyl terminus of the molecule (residues 396–415). The hydrophobicity of residues in this segment is plotted with positive numbers on the *y* - axis indicative of a hydrophobic nature and negative numbers representing hydrophilic sequences. (Reprinted, with permission, from Cool et al. 1990.)

The structure of the carboxy-terminal segment of TCPTP is summarized in Figure 2. The hydrophobicity plot indicates that this segment is predominantly hydrophilic, whereas the last 19 residues are hydrophobic and may contribute to the particulate localization of the 48-kD protein. A similar distribution of charged and hydrophobic residues is seen in the carboxy-terminal segment of PTP1B. The degree of conservation of sequence in these segments is much less than in the core catalytic domain; however, they are related, perhaps indicative of a general function in regulating activity and localization. Motifs within such segments, which are unique to a particular isoform, may point to specific roles for that enzyme. For example, the hydrophobic carboxyl terminus of TCPTP is preceded by a stretch of basic residues that possess the features of a nuclear localization signal (Cool et al. 1990). Whether it functions as such remains to be established. Clearly, it will be important to identify the putative targeting and regulatory proteins with which these low-M_r PTPases interact. The characterization of such interactions may illustrate a new tier of control of the level of cellular phosphotyrosine.

A general theme is becoming apparent among the family of PTPases. Distinct structural motifs within the molecule may control activity through interaction with targeting or regulatory proteins. Superimposed on this is the growing realization that the PTPases are themselves phosphoproteins. CD45 is known to be phosphorylated on Ser/Thr residues in vitro (Tonks et al. 1990) and in vivo (Autero and Gahmberg 1987). In fact, stimulation of lymphocytes with phorbol ester alters the surface distribution of CD45 (Patarroyo and Gahmberg 1984). In recent studies, it has also been shown that stimulation of Jurkat T cells with anti-CD3 antibodies or the mitogenic lectin phytohemagglutinin induces phosphorylation of tyrosyl residues in CD45 (Stover et al. 1991). The regulatory significance of this remains unclear. Although it may not affect activity directly, it may be of importance in controlling processes such as the redistribution of CD45 that accompanies T-cell activation (Minami et al. 1991). Phosphorylation of nontransmembrane PTPases on seryl, threonyl, and tyrosyl residues has also been observed (N.K. Tonks et al., unpubl.). It has recently been reported that stimulation of cAMP-dependent protein kinase or PKC led to an enhanced PTPase activity in the particulate fraction in various cell lines, although the precise mechanism of this effect remains to be elucidated (Brautigan and Pinault 1991).

Physiological Substrates for the PTPases

For a protein to be a substrate for a phosphatase, it must first have been phosphorylated by a kinase. Thus, the identification of the substrates for the tyrosine kinases and phosphatases essentially simplifies to the same problem. Many PTK substrates are only recognized by their apparent M_r on SDS gels, and the search for physiological substrates whose function is altered following tyrosine phosphorylation remains a major

goal of research in this area. Purified preparations of CD45 will dephosphorylate and inactivate phosphotyrosyl-MAP kinase in vitro (Anderson et al. 1990). Similarly, phosphotyrosyl-PLCγ is dephosphorylated and inactivated by TCPTP (Nishibe et al. 1990). These examples illustrate that the PTPases represent useful probes to discern the role of tyrosine phosphorylation, if any, in modulating the activity of a particular enzyme. However, the results do not necessarily reflect the specificity of the PTPases in vivo.

One approach to identifying the physiological role of CD45 has involved the generation of CD45-negative cells. Although one such T-cell line which is defective in surface expression of CD45 failed to respond to antigen or cross-linked CD3 but still proliferated in response to IL2, a $CD45^+$ revertant displayed the normal proliferative response to antigen and cross-linked CD3 (Pingel and Thomas 1989). Further experiments by Koretzky et al. (1990) have shown that $CD45^-$ HPB-ALL cells fail to generate phosphatidylinositol (PtdIns)-derived second messengers or elevate intracellular Ca^{++} in response to stimulation of the T-cell receptor. This was not due to a defect in the PtdIns turnover pathway itself, and the response to T-cell receptor stimulation could be restored by transfecting back cDNA encoding CD45. Similarly, in CD45-deficient Jurkat cells, signaling through the T-cell receptor and CD2 is abrogated, including the stimulation of PTK activity (Koretzky et al. 1991). These observations are important because they highlight the fact that CD45 may play a positive role in *stimulating* signal transduction events—thus, the PTPases should not be viewed simply as providing an "off-switch" to counter the activity of PTKs. The identity of the substrates involved remains to be established, but the products of the *lck* and *fyn* genes, members of the *src* family of PTKs, are likely candidates. These kinases possess a site of tyrosine phosphorylation in their carboxyl terminus that is inhibitory (for review, see Eisenman and Bolen 1990); dephosphorylation of this site by CD45 would be expected to activate the kinase. There is already circumstantial evidence to implicate $p56^{lck}$ as a substrate for CD45 (Ostergaard et al. 1989; Mustelin and Altman 1990). These cell lines not only represent a powerful tool to identify the substrates for CD45, but also provide a model system with which to analyze structure/function relationships in this receptor-PTPase.

A tyrosine-phosphorylated protein that represents a major focus of effort in the context of this meeting is the eukaryotic cell cycle regulator, $p34^{cdc2}$. In G_2 phase of the cell cycle, $p34^{cdc2}$ is phosphorylated on a tyrosyl residue in the ATP-binding site, and its activity is suppressed. Dephosphorylation of $p34^{cdc2}$ activates it as a histone H1 kinase and drives the cell from G_2 into mitosis (for review, see Lewin 1990). Consequently, the PTPase that dephosphorylates $p34^{cdc2}$ is a key element in the control of the cell cycle, and its identification and characterization will be an essential step toward our understanding of this process.

The timing of mitosis depends on a regulatory network of genes that act through $p34^{cdc2}$. The inhibitory pathway contains the *nim1*, *wee1*, and *mik1* gene products, which are protein kinases (Lundgren et al. 1991). In addition, there is a stimulatory pathway that contains $p80^{cdc25}$ (Russell et al. 1989). Strains of *S. pombe* bearing a temperature-sensitive mutation in *cdc25*, termed *cdc25*-22, arrest in G_2 at the restrictive temperature with $p34^{cdc2}$ phosphorylated on tyrosyl residues. Upon returning to the permissive temperature, there is dephosphorylation of $p34^{cdc2}$, and the cells advance into mitosis (Gould and Nurse 1989). Arrest at the restrictive temperature can be bypassed by expression of a form of $p34^{cdc2}$ in which the phosphorylated tyrosine has been mutated to phenylalanine (Gould and Nurse 1989) or by expression of the PTPase, TCPTP (Gould et al. 1990). In both cases, the cells advance prematurely into mitosis at a reduced cell size, the "wee" phenotype, and in the case of expression of TCPTP, this correlates with the dephosphorylation of tyrosyl residues in $p34^{cdc2}$. Thus, $p80^{cdc25}$ is intimately associated with the dephosphorylation and activation of $p34^{cdc2}$, but is it a PTPase?

Following the identification by Guan et al. (1991a) of the vaccinia virus protein VH1 as a distant relative of the PTPases, many investigators detected a somewhat limited structural similarity between VH1 and $p80^{cdc25}$ (Moreno and Nurse 1991). Although it will dephosphorylate and activate $p34^{cdc2}$ directly in vitro (Strausfeld et al. 1991), VH1 appears to display a restricted substrate specificity (of the substrates tested, only $p34^{cdc2}$ itself is dephosphorylated at a significant rate) and poor catalytic efficiency relative to TCPTP. However, given the distant relationship between $p80^{cdc25}$ and the other PTPases, it is entirely possible that the former utilizes a different catalytic mechanism, perhaps involving a stoichiometric association with the substrate $p34^{cdc2}$. Alternatively, an activating component may be missing in these in vitro reactions. Whereas in *S. pombe* dephosphorylation of Y15 in $p34^{cdc2}$ is sufficient for activation, in higher eukaryotes the adjacent threonyl residue, T14, is also phosphorylated (for review, see Lewin 1990). Thus, under these conditions, activation of $p34^{cdc2}$ should require either the coordinate action of tyrosine-specific and threonine-specific phosphatases or the action of a single broad specificity enzyme with the capacity to dephosphorylate both tyrosyl and threonyl residues. Interestingly, unlike the previously characterized PTPases, VH1, to which $p80^{cdc25}$ shows most similarity, displays dual specificity in vitro (Guan et al. 1991a). Whether $p80^{cdc25}$ functions as such in vivo remains to be established.

Identification of Novel PTPase Isoforms

In our laboratory, we have utilized a PCR-based strategy to identify the complement of PTPases in HeLa cells. Multiple PTPase-related sequences were amplified from a HeLa cell cDNA library including the known enzymes PTP1B, TCPTP, receptor PTPα, and LAR. In addition, three distinct PTPases were iden-

tified, two receptor-like molecules and a nontransmembrane PTPase (Yang and Tonks 1991) whose structure suggests it will be important in the control of cytoskeletal function (Fig. 3).

The latter cDNA encodes a protein of approximately 104 kD termed PTPH1, which can be described in terms of three segments: (1) The amino-terminal segment displays homology with amino-terminal domains in the cytoskeletal-associated proteins, band 4.1, talin, and ezrin; (2) the central segment did not show homology to sequences in the data base but did bear a number of putative phosphorylation sites for Ser/Thr kinases, including casein kinase II and $p34^{cdc2}$; (3) the segment displaying homology with members of the PTPase family is located at the carboxyl terminus. It should be noted that cDNA for a closely related PTPase was also isolated recently by another group (Gu et al. 1991). In collaboration with D.E. Hill and A. Bruskin, PTPH1 has now been expressed in BSC40 cells using the vaccinia virus expression system, and intrinsic PTPase activity has been demonstrated (N.K. Tonks et al., unpubl.). In a manner similar to the low-M_r enzymes PTP1B and TCPTP, activity against RCM lysozyme is stimulated by limited trypsinization, whereas the enzyme dephosphorylates MBP without prior proteolysis.

Amino acid and cDNA sequence analyses previously defined a family of proteins that encompassed band 4.1 (Conboy et al. 1986), talin (Rees et al. 1990), and ezrin (Gould et al. 1989; Turunen et al. 1989). In erythrocytes, band 4.1 promotes the association of actin and spectrin and through its amino-terminal segment interacts with the transmembrane protein glycophorin (Bennett 1989). Thus, it acts as a linker to anchor the cytoskeleton to the plasma membrane. Similarly, talin interacts with the transmembrane extracellular matrix (ECM) receptor, integrin. Ezrin displays a submembraneous localization in brush border cells, but the details of its interaction with other proteins remain to be established. Nevertheless, the homologous domain of approximately 340 residues at the amino terminus of these molecules appears to define a family of proteins that are targeted to the interface between the plasma membrane and the cytoskeleton. Therefore, we propose that PTPH1 will display a similar localization. Again, PTPH1 appears to be illustrative of the general theme among members of the PTPase family; distinct structural motifs within the protein may, at least in part, control specificity by restricting intracellular localization.

Transformation by oncogenic protein tyrosine kinases such as src is accompanied by dramatic alterations in cellular morphology; the cells assume a rounded, less adherent phenotype. This is associated with a disruption of the actin cytoskeleton and a reduction in the number of cell:cell and cell:substratum contacts (Kellie et al. 1991). Cells in culture adhere to the external substrate through specialized regions of the plasma membrane termed focal adhesion plaques. Within focal adhesions, the integrins provide the transmembrane link between the ECM and the cytoskeleton (Burridge et al. 1988). Their intracellular segments interact with actin through a multiprotein complex comprising talin, vinculin, α actinin, and undoubtedly other components yet to be described. Similar but less well characterized structures have been implicated in points of cell:cell contact. A contributing factor to the generation of the transformed phenotype is the phosphorylation of tyrosyl residues in several focal contact proteins including vinculin, talin, ezrin, paxillin, and the β subunit of integrin (Kellie et al. 1991). Connexin43 in adherens junctions has also recently been identified as a substrate for src (Swenson et al.

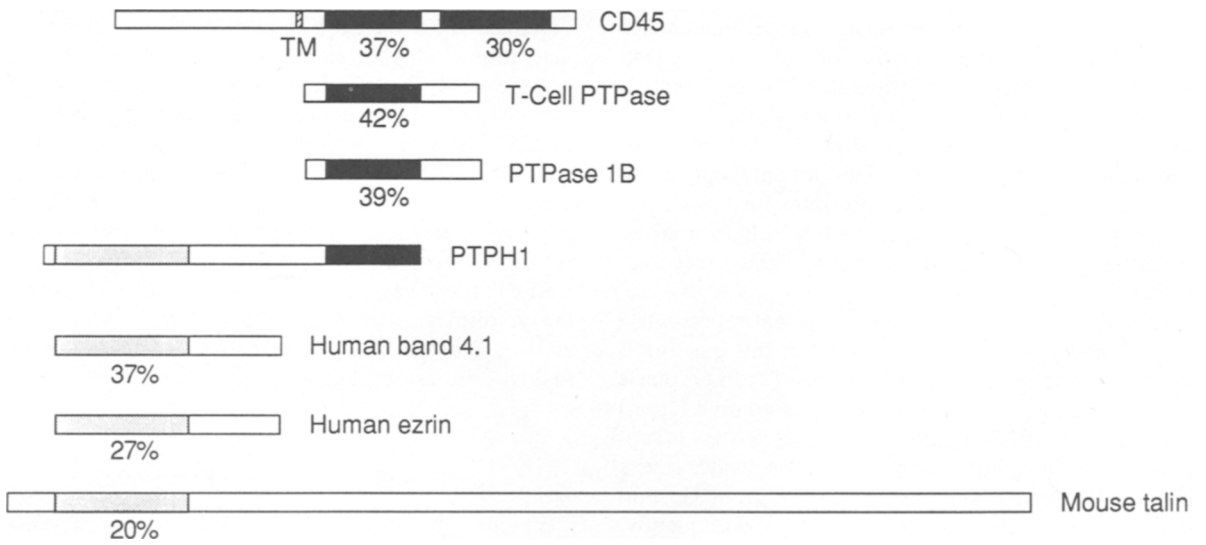

Figure 3. Structural organization of PTPH1. Shaded boxes denote the homologous segments in PTPH1 and the cytoskeletal-associated proteins, band 4.1, ezrin, and talin, which may direct these proteins to the interface between the plasma membrane and the cytoskeleton. The PTPase domains are shown in black. Homology among domains is indicated by the percentage identity. (Adapted from Yang and Tonks 1991.)

1990). The exact role of tyrosine phosphorylation in generating the transformed morphology remains unclear, although the phosphorylation of integrins may be crucial (Horvath et al. 1990). Immunocytochemical staining with antibodies to phosphotyrosine has indicated that phosphorylation of tyrosyl residues in focal adhesion and apical junction proteins also occurs in nontransformed cells (Maher et al. 1985), suggesting that PTKs, as yet to be identified, act on these sites during normal cell function. In view of the disastrous effects of aberrant tyrosine phosphorylation, one would anticipate that the activity of these PTKs will be tightly controlled. The localization of a PTPase to these structures is one means by which such regulation may be achieved. Studies are currently in progress to ascertain whether PTPH1 may serve such a role.

If PTPH1 is targeted to structures such as focal adhesion plaques, one would anticipate that following overexpression, it may be able to antagonize the action of src. PTPH1 was expressed constitutively from an LTR promoter in src-transformed NIH-3T3 cells. The expression vector also carried a neo-resistance gene, therefore, stable transfectants were selected in G418. Upon comparison of the src-transformed cells transfected with vector alone and those transfected with PTPH1 cDNA, a flat adhesive morphology, characteristic of nontransformed cells, was evident in the latter case. In addition, transfection with PTPH1 cDNA blocked focus formation, anchorage-independent growth, and tumor formation in nude mice. The level of cellular phosphotyrosine was also reduced in these cells relative to those displaying the transformed morphology. Such studies should provide a starting point from which to pursue the role of tyrosine phosphorylation in transformation induced by src. PTPH1 may prove to be a powerful probe to define precisely the key substrates of src involved in generating the transformed phenotype and to help identify the proteins phosphorylated on tyrosyl residues in the alterations in the cytoskeleton that accompany the normal course of the cell cycle. In view of the structural diversity observed within the known repertoire of PTPases, one might anticipate functional specificity in vivo. Although specificity among the PTPases may also be a feature of their capacity to revert transformation induced by particular PTKs, this hypothesis remains to be tested exhaustively.

PERSPECTIVES

One area in which rapid progress is anticipated is the development of the concept that the PTPases may function as antioncogenes. The increase in tyrosine phosphorylation observed in some transformed cells may be achieved by inactivation of a PTPase rather than by activation of a PTK. Treatment of NRK-1 cells with vanadate, which among other actions is an inhibitor of PTPases, enhanced the level of cellular phosphotyrosine and led to the production of a transformed morphology (Klarlund 1985). Examination of the chromosomal localization of various PTPase genes is highlighting some interesting correlations with sites of abnormality associated with certain neoplasias. Most notably, the gene for receptor PTPaseγ maps to a region of human chromosome 3, 3p21, that is frequently deleted in renal cell and lung cell carcinomas. In three of the lung tumors, one RPTPγ allele was lost whereas flanking loci were not deleted (LaForgia et al. 1991). Similar studies with the gene for PTP1B also appear interesting (Brown-Shimer et al. 1990).

How many PTPases are there? Despite the recent explosion in the number of members of the family to be identified, it seems safe to assume that we are only viewing the tip of a very large iceberg. It is likely that the PTPases will rival the family of PTKs in their structural diversity and complexity. Using PCR protocols, 27 PTPase-related sequences were identified in the protochordate, *Styela plicata* (Matthews et al. 1991). Furthermore, we have identified 15 distinct sequences in a *Xenopus* oocyte cDNA library and have confirmed the presence of mRNA for each species (Q. Yang and N.K. Tonks, in prep.). Obviously, there exists considerable complexity in the pattern of expression of PTPases even within a single cell. It is hoped that the characterization of these species will lead us to a more sophisticated understanding of the physiological role of tyrosine phosphorylation.

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