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Structure and Catalytic Properties of Protein Tyrosine Phosphatases^a

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The level of protein tyrosine phosphorylation is controlled within the cell by both tyrosine kinase and phosphatase activities. Our laboratory has focused its energy on the protein tyrosine phosphatases (PTPases). To understand the latter, we and others have cloned a number of PTPases.^{1,2} Now, more than 40 cDNAs are known that encode a wide spectrum of PTPases. The PTPases can be subdivided into two major families. One family of the PTPases resembles receptors: the PTPases have an intracellular tyrosine phosphatase domain(s), a transmembrane domain, and an extracellular domain that may function in cell-cell or ligand-cell interactions.^{1,2} This family has been further classified into five subtypes based largely upon differences in their extracellular domains (FIG. 1). We have a limited understanding of the potential ligands for the receptor-like PTPases. However, the sequence similarity of type II PTPases to cell adhesion molecules (CAM) suggests that these PTPases may exhibit homophilic binding. Expression of PTP μ in SF9 insect cells results in cell aggregation, suggesting that the extracellular domains of PTP μ mediate this self-aggregation.^{3,4} The expression of cytoplasmic domain-deleted constructs indicates that PTPase catalytic activity is not required for adhesion; only the extracellular domain is necessary for cell-cell interactions. The purified extracellular domain conjugated to beads can also mediate bead-bead adhesion. Work from Schlessinger's laboratory also indicates that the closely related molecule PTP κ also displays homophilic adhesive properties.⁵ When cells expressing PTP μ are incubated with cells expressing PTP κ , the cells segregate and adhere in a homophilic fashion. This shows that, although PTP μ and PTP κ are structurally similar, they display a high degree of specificity in their cell-cell adhesion.

The second family of PTPases is found exclusively within a cell.^{1,2,6} The distinguishing feature of the intracellular PTPase is the diversity of sequences flanking a single catalytic domain (FIG. 2). One of the functions of these flanking sequences appears to be the targeting of the enzyme to specific intracellular locations. These include (1) membrane-association domains, (2) nuclear-localization domains, (3) Src homology 2 (SH2) domains, and (4) cytoskeletal-association domains. Locating specific PTPases to define subcellular locations will undoubtedly restrict and define their substrate specificity.⁶

^aThis work was supported by the National Institutes of Health (NIDDKD 18024) and the Walther Cancer Institute.

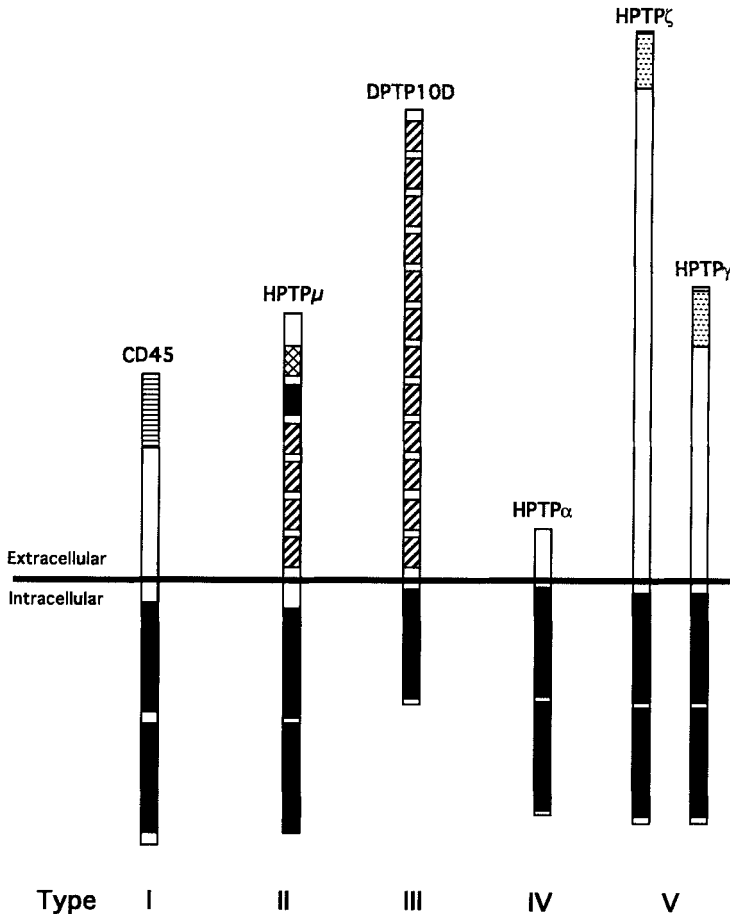


FIGURE 1. Transmembrane receptor-like PTPases. The members of this family of PTPases possess a single transmembrane domain and one or two intracellular PTPase catalytic domains (black bar) (see ref. 16 for details). They can be subdivided into five types on the basis of their extracellular domain structures: I, CD45; II, HPTP μ ; III, DPTP10D; IV, HPTP α ; and V, HPTP ζ and HPTP γ . The extracellular domain structures are shown: amino terminus isoforms (horizontal lines) resulting from differential splicing; immunoglobulin-like (vertical lines); fibronectin type III-like (shaded bar); Meprin amino acid motif (MAM) adhesive protein homology-like (diagonal lines); and carbonic anhydrase-like (stippled). (Mauro & Dixon.⁶ With permission from Elsevier Trends Journals.)

We discovered that certain pathogenic bacteria also have a protein that has protein tyrosine phosphatase activity. This is unusual because bacteria are not known to contain proteins that are phosphorylated on tyrosine. The bacteria that have the tyrosine phosphatase activity are from the genus *Yersinia*. This bacterium was responsible for the plague or the Black Death that reduced the population of Europe on several

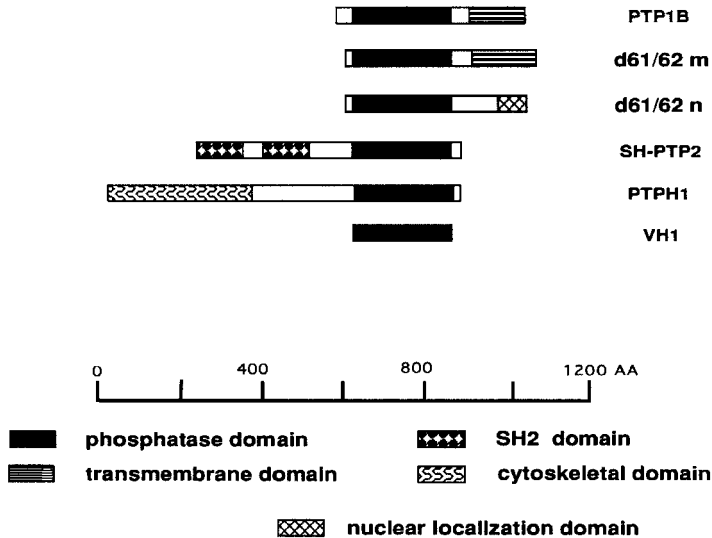


FIGURE 2. Structural features of representative intracellular PTPase. Each PTPase possesses one conserved catalytic domain. The PTPase pictured with their corresponding noncatalytic sequences include (a) membrane-association domains in PTP1B; (b) an Src homology 2 (SH2) domain in SH-PTP2; and (c) cytoskeleton-association domains in PTPH1. (Mauro & Dixon.⁶ With permission from Elsevier Trends Journals.)

occasions by more than 25 million people. As you are well aware, the plague has recently broke out again in India. This simply points out that such diseases, although treatable by antibodies, have not been eliminated as human pathogens. We have shown that the *Yersinia* PTPase is a virulent gene in bacterium and that it functions to dephosphorylate tyrosine residues necessary for regulating mechanisms within the mammalian host.⁸

We also observed that the *vaccinia* virus and other members of the pox virus family (including small pox) have encoded within their genomes a phosphatase. The activity of the *vaccinia* phosphatase is not restricted to the dephosphorylation of tyrosine; it will also dephosphorylate serine- and threonine-containing substrates.⁹ The *vaccinia* phosphatase has an amino acid sequence identity to the yeast cell cycle gene product, *cdc 25*, and a group of related mammalian dual-specific phosphatases. TABLE 1 lists representatives of the various dual-specific phosphatases that have been described to date.¹⁰

All PTPases possess at least one 230 amino acid catalytic domain containing a highly conserved active-site region with the consensus motif [I/V]HCXAGXXR[S/T]G (where X is any amino acid). This region is the signature sequence of most PTPases.^{11,12} Site-directed mutagenesis and trapping experiments have shown that the cysteinyl residue within this motif is essential for phosphatase activity. The enzyme appears to form a thiol-phosphate intermediate during catalytic turnover.¹³ Interestingly, this catalytic domain of the PTPases bears no resemblance to that of the serine-threonine phosphatases or of the alkaline or acid phosphatases. Recently,

TABLE 1. The Dual-specific PTPases

Dual-specific PTPases	Substrate Motif	Induction	Function
HVH1, CL100, SCH134, MKP-1	-(pT)E(pY)-	Serum, growth factors, phorbol esters	Deactivation of ERK ^a , MAP ^b kinase
PAC1	-(pT)E(pY)-	T-cell activation	Deactivation of ERK-MAP kinase
Cdc25	-(pT)(pY)-	Cell cycle-dependent	Activation of Cdc2; entry into mitosis
KAP, Cdi1	-(pT)(pY)-	Cell cycle-dependent	Cell-cycle regulation; associates with CDK2, cdc2
YVH1	—	Nitrogen starvation	Growth control
MSG5	-(pT)E(pY)-	Mating pheromone	Inactivation of FUS3: adaptation to pheromone response in yeast

^a Extracellular signal-regulated kinase.

^b Mitogen-activated protein.

the structures of two PTPases have been solved.^{14,15} Their structures are quite similar and support the idea that the PTPase most likely employs a common catalytic mechanism. Details of the structure and the catalytic mechanism will be discussed.

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