Protein tyrosine phosphatases in disease processes

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Given the importance of tyrosine phosphorylation of proteins in signalling pathways, it is perhaps not surprising that protein tyrosine phosphatases (PTPs) are involved in the pathogenesis of certain human diseases. A PTP produced by the Yersinia bacteria (which can cause bubonic plague, septicemia and enteric diseases) is thought to be used as a 'weapon' against host cell functions. In addition, dysfunction of cells' endogenous PTPs may contribute to defective immune function, to cancer and to diabetes.

The phosphorylation of signalling proteins on tyrosine is essential for cellular regulation of growth and differentiation. Many oncogenes and their cellular homologues are protein tyrosine kinases (PTKs), and excessive tyrosine phosphorylation has been correlated with cellular transformation[1,2]. The level of tyrosine phosphorylation in the cell is also modulated by specific protein tyrosine phosphatases (PTPs). In many cases we know that the activity of individual PTPs responds to signals of growth and development but we are only beginning to understand how these enzymes initiate or modulate cellular responses to these signals.

The PTPs are a family of enzymes united by a common catalytic core domain but containing diverse attached sequences devoted to the regulation of activity, substrate recognition, or localization to subcellular compartments[3–5] (Fig. 1). The catalytic core is ~250 amino acids long, and contains ~21 invariant residues, many of which have been shown to be necessary for phosphatase activity[6]. Within the core domain is the active-site signature motif [I/V]HCxAGxxR[S/T]G (in the one-letter amino acid code where x can be any amino acid). The cysteine within this sequence is required for catalytic activity, and is involved in the formation of a phosphoryl–enzyme intermediate[6]. Sequences outside the catalytic core of the PTP include Src homology region 2 (SH2) domains (which modulate phosphatase interactions with other proteins) extracellular ligand-binding domains, and signal sequences that direct PTPs to the cytoplasmic membrane, the nucleus, and other compartments. Although all PTPs recognize phosphotyrosine-containing proteins as substrates, a subset, the dual-specific phosphatases, recognizes proteins phosphorylated on both tyrosine and serine/threonine. The family affiliations and proposed functions of PTPs discussed in this article are outlined in Table 1.

The *Yersinia* PTP and its role in pathogenicity

Three species of the bacterial genus *Yersinia* are responsible for disease in humans[7,8]. *Y. pestis* is the cause of bubonic plague, while *Y. enterocolitica* and *Y. pseudotuberculosis* cause a variety of enteric illnesses and septicemia. *Yersinia pestis* enters the dermal lymphatics through the bite of an infected flea. The enteropathogenic *Yersiniae* traverse the epithelial cell layer in the small intestine and colonize the Peyer's patch, a specialized lymphoid follicle. The bacteria encounter and evade destruction by lymphocytes and macrophages and replicate extracellularly in the reticuloendothelial system.

The enteropathogenic *Yersiniae* can be internalized into epithelial cells by three known routes, one of which, invasin-mediated uptake, is the best understood[7,8]. The chromosomal *inv* gene encodes the protein invasin, which mediates binding of bacteria to

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TABLE 1 – FUNCTION OF PTPs

PTP	Family	Function
Yersinia YopH	PTP	Dephosphorylation of unknown substrates in host phagocytes; essential for bacterial pathogenicity.
Vaccinia VH1	Dual-specific PTP	Dephosphorylation of unknown substrates in mammalian cells; expressed late in viral infection.
HCP	SH2-domain PTP	Dephosphorylation of unknown substrates in cells of haematopoietic origin; mutation leads to abnormal development of these cells in mice, and possibly immunological dysfunction in humans.
csw	SH2-domain PTP	Dephosphorylation of unknown substrates during *Drosophila* development; transduces signals from the torso receptor; mutation leads to morphological abnormalities.
SH-PTP2/Syp	SH2 domain PTP	Dephosphorylation of activated IRS-1 and other, unknown substrates; association with activated growth factor receptors, where it may also provide an adapter function within signalling complexes; postulated role in normal insulin signalling and growth control; no known human genetic diseases caused by its mutation have been identified.

host cell β_1 integrins. The cytoplasmic domains of these receptors are in contact with the cytoskeleton at focal adhesions[9]. It is thought that PTK activity induced by integrins upon ligand (invasin) binding triggers cytoskeletal reorganization, resulting in phagocytosis of the bacterium by epithelial cells[8].

Yersiniae neutralize phagocytes such as macrophages by attaching to them and then, by a mechanism that is not understood, secreting virulence determinants, called Yops, into these cells[10]. Yops are encoded by a 70 kb virulence plasmid. Of the ten or eleven Yop proteins, one, YopH (also known as Yop2b and Yop51) is a PTP. YopH is essential for virulence: deletion of the gene in *Y. pseudotuberculosis* impairs the ability of the bacteria to resist phagocytosis[11]. The YopH PTP is the most active PTP yet isolated[6]. The importance of this activity to bacterial virulence is underscored by the fact that mutation of the active-site cysteine to alanine renders the PTP inactive and the bacterium avirulent[12].

The targets of YopH are unknown, but may include tyrosine-phosphorylated proteins involved in the cytoskeletal reorganization that leads to phagocytosis, as well as phosphoproteins important in signal transduction. *Yersinia* infection of cultured macrophages results in reduction of the level of phosphotyrosine on many cellular proteins[12]. Two putative YopH substrates, a 120 kDa and a 55 kDa phosphoprotein, have been identified[12,13]. The phosphotyrosine levels of the 120 kDa protein decrease during the course of *Yersinia* infection of a macrophage cell line[13]. This effect is dependent on a functional YopH protein, since the phosphorylation level of this protein is constant in the presence of a catalytically inactive YopH mutant (in which the active-site cysteine has been replaced by alanine). The catalytically inactive mutant protein was used to coimmunoprecipitate both the 120 kDa and 55 kDa proteins. These proteins each autophosphorylate on tyrosine *in vitro*. Their identities remain to be determined.

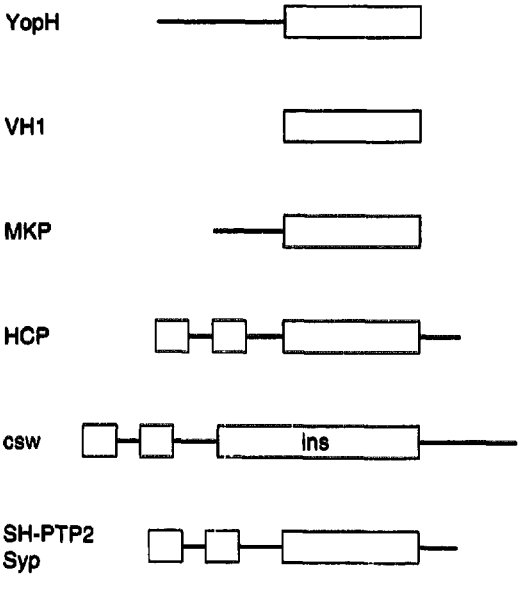

FIGURE 1

Domain structure of PTPs. The core PTP domain is shown as a shaded rectangle. Some PTPs have unique N- or C-terminal extensions. SH2 domains – 100 amino acid modules that recognize phosphotyrosine (in the same protein or other protein) in the context of surrounding sequences[48] – are shown as small squares. The SH2 domain may serve to localize a PTP to a specific compartment within the cell or may also function to regulate activity. csw and SH-PTP2/Syp have the same SH2–SH2–PTP structure as HCP but they are more closely related to each other than to HCP and are considered homologues[40]. In addition, csw, but not SH-PTP2/Syp, has a 150 amino acid serine- and cysteine-rich insert (ins) of unknown function in its PTP domain[41].

The vaccinia virus PTP: impact on the cell cycle?

The viral genus *Orthopoxvirus* includes members that cause smallpox. These viruses, now eliminated as source of disease, enter the host through the upper respiratory tract, are drained by the lymphatics to the lymph nodes and enter the bloodstream[14]. They invade the reticuloendothelial system and then the epidermis, causing skin eruptions. Further consequences of viral infection include general toxemia, septic shock, intravascular coagulation, and death. One relatively benign member of this viral genus, vaccinia, has been studied extensively. Vaccinia virus is a large (192 000 bp) double-stranded DNA virus, containing ~200 genes, that replicates in the cytoplasm of the host cell[15]. In addition to encoding an impressive array of enzymes concerned with viral DNA replication and transcription, the virus encodes many proteins that inhibit host cell DNA, RNA and protein synthesis and defend the virus against the host immune system.

Vaccinia virus encodes a small (20 kDa) PTP of unknown function called VH1 (for vaccinia *Hin*dIII fragment H, ORF 1) that is produced late in infection[16]. This PTP is unusual in that it contains only the active-site signature sequence, with no readily identifiable regulatory sequences[16,17] (Fig. 1). The VH1 gene is highly conserved among the orthopoxviruses[18] and this fact, along with the compact nature of the viral genome, makes it likely that VH1 plays an important role in the viral life cycle. The protein substrate(s) of VH1 are unknown.

VH1 is the prototype of a family of PTPs that can dephosphorylate both phosphotyrosine- and phosphoserine/threonine-containing substrates[17]. Other dual-specific phosphatases have been discovered recently and many of them are involved, directly or indirectly, in regulation of the cell cycle. The active-site region of VH1 is similar to that of the mitotic regulator cdc25, which dephosphorylates the cdc2 kinase on Thr14 and Tyr15 to allow entry into mitosis[19,20]. PTPs that are induced in response to mitogen stimulation (PAC1, MKP-1), oxidative stress and heat shock (CL100) in mammalian cells, and mating factor (MSG5) and nitrogen starvation (YVH1) in yeast[21–25],

also appear to be dual-specific. PAC-1, MPK-1, CL100 and MSG5 dephosphorylate mitogen-activated protein (MAP) kinases[24,26–28]. MAP kinases are a family of serine/threonine kinases activated by phosphorylation on conserved threonine and tyrosine residues in response to a variety of growth factors and hormones[29,30]. They are key players in signal transduction cascades that link receptor protein tyrosine kinases to nuclear transcription factors in mammalian cells and to the transcriptional and cell cycle machinery in yeast. Depending on the stimulus, cells may undergo either cell cycle progression or arrest. The dual-specific phosphatases may regulate these pathways by preventing either excessive growth or prolonged quiescence. It is tempting to speculate that VH1 interferes with the cell cycle by competing with an endogenous dual-specific phosphatase for its substrate. Further investigation is needed in order to identify changes effected at both the cellular and molecular levels by VH1.

Endogenous PTPs in cell growth and developmental regulation

Although no human diseases have been clearly ascribed to defective PTP function, studies of the mouse haematopoietic cell phosphatase (HCP) and *Drosophila* corkscrew (csw) have shown that aberrant patterns of growth and development may occur when normal copies of cellular PTPs are mutated[31]. In addition, as discussed below, mammalian homologues of csw are found to be associated with activated growth factor receptors[32–34], as well as insulin receptor substrate-1 (IRS-1)[35]; the study of these interactions may shed light on diseases as diverse as cancer and diabetes.

HCP and the csw family of PTPs are cytoplasmic proteins that have a common structure, consisting of two N-terminal SH2 domains, a single central PTP domain, and a C-terminal tail (Fig. 1). HCP (also known as SH-PTP1 and PTP1C) is a 68 kDa protein produced in a variety of mouse and human primary haematopoietic cells and cell lines[36]. Mice homozygous for the recessive motheaten (*me*) allele, or the less severe *me*^{v} allele, carry mutations in the gene encoding HCP and have a variety of immune cell deficiencies[37]. These include defects in lymphocyte, natural killer cell, and macrophage development and/or function, leading to death after several weeks to a few months. The *me* and *me*^{v} mutations are single base-pair changes that cause aberrant splicing of the HCP mRNA[37–39] and synthesis of an out-of-frame, truncated PTP in *me/me* mice, or lead to proteins with small deletions or insertions of amino acids in *me*^{v}/*me*^{v} mice. In both cases, HCP enzyme activity in macrophages is reduced. The human HCP gene maps to chromosome 12p12-p13, a region that is associated with rearrangements in 10% of cases of childhood acute lymphocytic leukaemia. Other diseases that might originate from defects in HCP are several types of familial immunological dysfunctions, whose clinical manifestations are similar to those displayed by *me/me* or *me*^{v}/*me*^{v} mice (reviewed in Ref. 37).

In contrast to HCP, which is produced primarily in cells of haematopoietic lineage, the *Drosophila* corkscrew (csw) protein and its human (SH-PTP2) and mouse (Syp) homologues appear to be produced ubiquitously in different tissues and throughout development[34,40,41]. However, csw has a specific role in *Drosophila* development: *csw* mutants form inviable zygotes with abnormal development of their terminal (head and tail) structures[41].

Several lines of evidence suggest that csw and its mammalian relatives interact with activated growth factor receptors: genetic epistasis experiments indicate that during development csw acts downstream of the torso receptor (a homologue of the PDGF receptor), and in concert with the *Drosophila* Raf protein, to transduce signals to downstream transcription factors[41]; SH-PTP2/Syp coimmunoprecipitates the EGF and PDGF receptors, with association increasing after growth factor stimulation[33,34]; and a phosphotyrosyl peptide of the PDGF receptor (comprising the SH-PTP2/Syp-binding site as defined by peptide competition experiments) was found to stimulate the activity of SH-PTP2/Syp five- to tenfold[42].

It is not clear whether the growth factor receptors are substrates for these PTPs since the EGF receptor was not dephosphorylated by SH-PTP2/Syp under conditions where SH-PTP2/Syp was active in dephosphorylation of an artificial substrate[34]. Rather, binding of these PTPs to activated growth factor receptors via SH2 domains may be a prerequisite for downstream signal transduction events. Before growth factor stimulation, SH-PTP2/Syp is phosphorylated predominantly on threonine and serine; after stimulation, phosphorylation is found on tyrosine[33]. These results suggest a model in which the PTP associates with an activated, tyrosine-phosphorylated growth factor receptor via SH2 domains, is phosphorylated on tyrosine, and then itself becomes a potential target for binding of other SH2-domain proteins, possibly substrates. Evidence in support of this model is that PDGF stimulation induces the formation of a complex containing the PDGF receptor, Sos (a guanine-nucleotide-exchange factor that activates Ras), Grb2 (an adapter protein that links Sos and Ras), and SH-PTP2/Syp[43]. In this complex, SH-PTP2/Syp joins Grb2 to the PDGF receptor. Thus at least one of the signalling functions of SH-PTP2/Syp may be as an adapter, coupling PDGF receptor activity to Ras activation of the kinase cascade that in turn leads to cellular proliferation.

In addition to an involvement in growth factor signalling, SH-PTP2/Syp is involved in insulin signalling. Insulin binding causes the insulin receptor to autophosphorylate on tyrosine, and then the activated receptor phosphorylates substrate proteins on tyrosine[44]. One of these, IRS-1, binds SH2-domain proteins that dock at multiple tyrosine-phosphorylated sites. In adipocytes treated with insulin, SH-PTP2/Syp was found to be associated with IRS-1[35]. Association between SH-PTP2/Syp and IRS-1 leads to the rapid dephosphorylation of IRS-1[45]. Specific phosphotyrosyl peptides from IRS-1 stimulated the PTP activity of SH-PTP2/Syp towards artificial substrates up to 50-fold[46]. Surface plasmon resonance studies revealed

that other phosphotyrosyl peptides from IRS-1 bound strongly to either the N-terminal or C-terminal SH2 domain from SH-PTP2/Syp[46]. These data suggest that each SH2 domain can bind to a distinct phosphotyrosine residue of IRS-1 while activating hydrolysis of a third phosphotyrosine residue at the active site.

Why is SH-PTP2/Syp associated with IRS-1 and what is its role in insulin signalling? When tyrosine phosphorylation of the insulin receptor is stopped by permeabilization of adipocytes in the presence of EDTA, IRS-1 is rapidly dephosphorylated[47]. Dephosphorylation of IRS-1 might be part of the mechanism responsible for the cessation of signalling in response to insulin withdrawal. Alternatively, SH-PTP2/Syp might act in an analogous way to csw, which transduces the signal emanating from the torso receptor in a positive manner. If this is the case, dephosphorylation of IRS-1 or other substrates might be necessary for propagation of the insulin-induced signal.

Future directions

The studies described in this article represent a start towards understanding the role of PTPs in disease processes. At the same time, they are providing insight into the basic biology of the regulatory networks that are affected by PTPs. In order to understand what roles PTPs are playing, two major questions remain to be answered: what are the substrates for these phosphatases, and do the phosphatases function as early players in signal transduction pathways or do they function later in adaptation or response cessation? Answering these questions will increase our understanding of normal growth and development as well as the molecular basis of disease.

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