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PROTEIN TYROSINE PHOSPHATASES

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1. ABSTRACT

The molecular mechanisms of signal transduction have been at the focus of increasingly intense scientific research. As a result, our understanding of protein tyrosine kinase-mediated signaling has advanced at an unprecedented pace during the past decade. In contrast, the study of protein tyrosine phosphatases has lagged behind, but is now gathering momentum and is predicted to become a “hot topic” in the field within the next few years. This review summarizes the current state-of-the art in our understanding of the structure, regulation and role of protein tyrosine phosphatases with emphasis on the lymphocyte system.

2. INTRODUCTION

Phosphorylation of proteins on tyrosyl residues is a vital mechanism for many signal transduction pathways regulating cell growth, differentiation and development (1-3). Although the phosphotyrosine (PTyr) content of cellular proteins is the net result of the opposing effects of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPases), most researchers have concentrated on the PTKs, many of which were cloned and sequenced nearly two decades ago. The first PTPase was not cloned until 1990 and most PTPases have been known for much less than ten years. Although our progress has been remarkable, and clearly is accelerating, very little is still known about most of these enzymes.

The human genome has been reported to contain 56 PTPase genes (4), of which nearly 50 have been published in some form, plus 29 dual-specificity PTPases (4), of which nearly 20 are known. These numbers should be compared to the 106 human genes for protein tyrosine kinases (4). Despite ratio of a few more PTKs than PTPases, the level of PTyr in normal cells is only 0.1-0.01% of acid-stable protein-bound phosphate, or 3-4 orders of magnitude lower than the level of phosphoserine (PSer) and phosphothreonine (PThr), suggesting that the cellular PTPases readily counteract the PTKs in normal cells. Whether this is due to a high number of expressed PTPases, high expression levels of some PTPases, or high specific activity, is not yet clear. Importantly, even a small increase in steady-state tyrosine phosphorylation caused by deregulation or overexpression of some PTKs, notably members of the Src family (5), can induce acute malignant transformation and uncontrolled proliferation of cells. Also physiological processes mediated by tyrosine phosphorylation (e.g. growth factor signal transduction) are accompanied by relatively small and transient increases in cellular PTyr. Thus, it is clear that PTPases play an important role in the maintenance of normal cell physiology by counteracting substrate phosphorylation by the PTKs. To complicate matters, we and others have found that several PTPases directly regulate the PTKs, either negatively or positively (5-10), by dephosphorylating specific regulatory tyrosine phosphorylation sites in these PTKs. At the same time, some PTPases are also phosphorylated by the PTKs (11-20), indicating that PTK - PTPase interactions can be rather complex. There are several examples of PTPases that are regulated by phosphorylation on tyrosine, threonine or serine residues and therefore also must be dephosphorylated by themselves ('autodephosphorylation') or by other protein phosphatases, perhaps forming 'phosphatase cascades' analogous to the kinase cascades involved in many signal transduction processes.

Like most normal cells, resting lymphocytes contain very low levels of PTyr, presumably reflecting the finding that overall PTPase activity is many orders of magnitude higher than overall PTK activity (6). This conclusion is in agreement with the truly dramatic increase in intracellular tyrosine phosphorylation that follows a brief treatment of T cells with membrane-permeable PTPase inhibitors, such as phenylarsine oxide (21) or pervanadate (22,23). The increase in PTyr-content after addition of these pharmacological agents is detectable within seconds and rapidly reaches levels that far exceed the response to any physiological stimuli. These experiments emphasize the importance of PTPases in the maintenance of the resting levels of tyrosine phosphorylation. Since these inhibitors also cause many functional events of T cell activation (22,23) and prevent reversion of activated T blasts to a resting state (24), it seems that PTPases play a crucial part in maintenance of the resting phenotype of lymphocytes.

Lymphocyte activation is a convenient and popular model for studying signal transduction and mitogenesis. Consequently, the molecular mechanisms of antigen receptor-mediated signaling are relatively well understood (although many questions do remain unresolved). Upon activation of lymphocytes by ligation of their receptors for antigen or other mitogens together with appropriate co-receptors or accessory molecules, there is a very rapid, but transient, increase in PTyr in a number of cellular proteins (25-27, reviewed in 28,29). Our original observation (30) that inhibition of this response by pharmacological means blocks lymphocyte activation completely gave rise to the current dogma that the first event triggered by receptor ligation is the activation of one or several PTKs (5,30,31). Several PTKs are currently known to participate in the initiation of lymphocyte activation. Using homologous recombination, transgenic mice, and mutant cell lines, it has been demonstrated that two members of the Src family of PTKs, Lck and Fyn, are important (31-39) in T cells, although their relative contribution and redundancy remains a bit unclear. In B cells, the corresponding Src family PTKs are Lyn, Blk and Fyn, while natural killer (NK) cells also utilize c-Fgr (5). The two Syk family PTKs ZAP-70 and Syk are also recruited and activated within the first minute (28,40) and lack of a functional *zap* gene is known to cause a severe immunodeficiency in humans (41). TCR crosslinking also transiently activates the Csk kinase (42), which negatively regulates Lck and Fyn (43). Finally, the Tec family PTKs Btk and Itk/Emt (44,45) are activated in B cells and T cells, respectively. The importance of all these PTKs is also demonstrated by the transforming capacity of some of them (5) and the observed changes in amount or function of them in malignancies, *e.g.* in T cell lymphomas (46-48), and in T cell anergy (5).

The molecular mechanisms of signal transduction and lymphocyte activation have been intensely studied during past few years (5,49-51). It has been established which PTKs carry the main responsibility for initial signal transmission and these enzymes, their physiological importance, regulation, and substrates have been widely studied. In contrast, it is far from clear which PTPases are involved in lymphocyte activation, how they are regulated or what substrates they act on. In fact, it is likely that the most important PTPases have not yet been found. Although the main focus of most researchers has been on the PTKs, it is generally acknowledged that PTPases are likely to be equally important in the propagation and coordination of

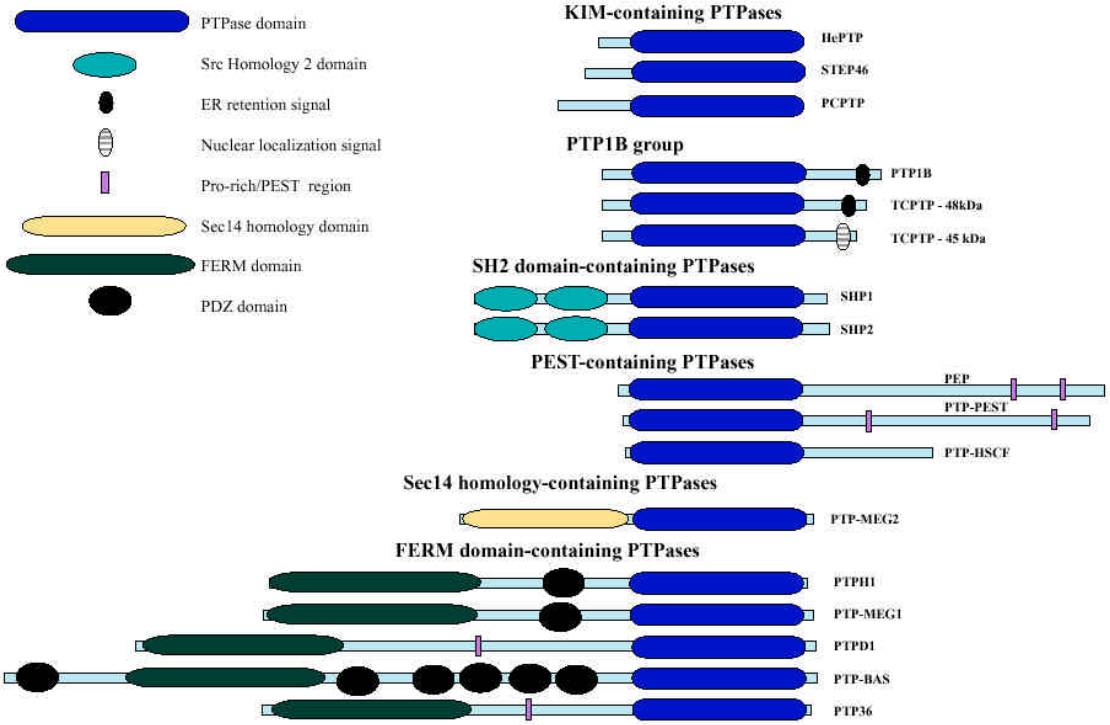

Figure 1. Structure of the intracellular classical PTPases. The enzymes are aligned for their ~260-amino acid PTPase domain and the location of different domains (key in upper left had corner) indicated. All PTPases are shown in the same scale, except PTP-BAS, which is shown shorter than its actual relative size.

the signaling cascades (52-56). We predict that there will be a sharply increasing interest in PTPases within the next few years, accompanied by many significant breakthroughs in our understanding of these enzymes and their roles in physiological processes. They will also become the focus of targeted drug discovery for the treatment of human diseases. This review will focus on all the PTPases that are expressed in lymphocytes. We will summarize and discuss their known, suspected or potential roles in lymphocyte physiology.

2.1. PTPases present in lymphocytes

The purification to homogeneity in 1988 of the first PTPase, PTP1B from human placenta (57,58), and the subsequent isolation of its cDNA in 1990 (59), provided the necessary basis for a search for related genes using a number of molecular biology techniques. The sequence of PTP1B also revealed that a well-known molecule, the leukocyte common antigen CD45, was a PTPase with two catalytic domains in tandem in its cytoplasmic tail (60). Thus it became clear from the very start that PTPases can be either transmembrane molecules or entirely intracellular enzymes, and that they can have more than one PTPase domain. During the early part of the 90s, a number of PTPases related to PTP1B and CD45 were discovered in eukaryotic cells from yeast to man (52-56). All these enzymes share several regions of homology, including a well-conserved signature sequence motif (HCXXGXXR). PTP1B was recently crystallized and its three-dimensional structure solved (61). The structure showed that the cysteine residue of the signature sequence resides at the bottom of a deep hydrophobic catalytic pocket and is a crucial component of the catalytic machinery (62). Specificity for PTyr is determined by the depth of the pocket (63). During catalysis the phosphate moiety is transferred from the substrate to the cysteine to form an enzyme-phosphocysteine intermediate, which is regenerated through the formation of free phosphate with the help of a water molecule (64).

The ”classical” PTPases comprises the largest family of PTPases and contains all currently known transmembrane enzymes, as well as PTP1B, and several related intracellular enzymes, all with a ~250 amino-acid catalytic domain with a relatively high degree of similarity. All intracellular members of this family have one copy of the catalytic domain, which can be located anywhere in the molecules. In contrast, many transmembrane PTPases have two PTPase domains in tandem in their intracellular C-terminal part. In many cases, the second domain appears to be enzymatically inactive and its function remains speculative. The classical PTPases can be further subdivided in subfamilies or groups based on overall structure and the presence of other domains (figure 1). For example, the group consisting of SHP1 and SHP2 is characterized by two SH2 domains in the N-terminal half of the molecule and a PTPase domain in the C-terminal half. Another group, often referred to as the “cytoskeletal”

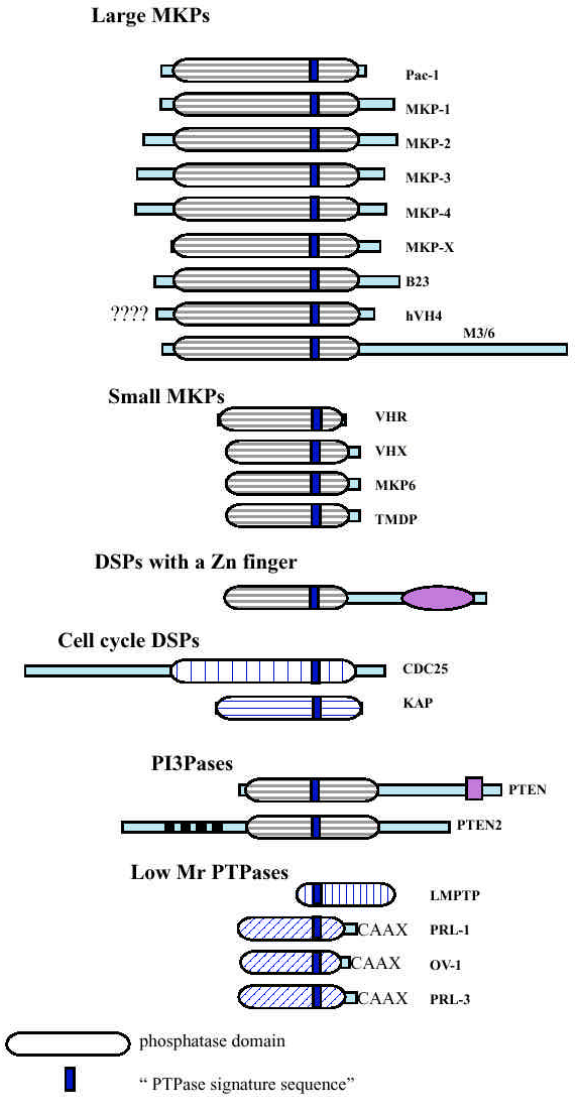

Figure 2. Structure of dual-specificity phosphatases and low-molecular weight PTPases. The enzymes are aligned for their "PTPase signature sequence". The extent and exact placement of the phosphatase domain is uncertain in many. We have included the CH2 domain in the phosphatase domain. A low degree of amino acid similarity to other enzymes is indicated by different shading of the domain. In PTEN, the phosphatase domain overlaps with a tensin homology region (in pink). CAAX, isoprenylation/farnesylation signal.

PTPases, is characterized by an N-terminal region of homology to band 4.1 and other cytoskeletal proteins (termed the ERM or FERM domain), a central region often with at least one PDZ domain, and a C-terminal PTPase domain. The PEST group consists of enzymes with an N-terminal PTPase domain and C-terminal region rich in proline, glutamic acid, serine and threonine. These enzymes will all be discussed in detail below.

In addition to the "classical" family of PTPases with a high degree of similarity to PTP1B and CD45, another family of enzymes that not only dephosphorylate PTyr, but also PThr, was discovered by K. Guan and J. Dixon (65). The founding member of this family of dual-specific protein phosphatases was the VH1 protein from Vaccinia virus (65). A closely related enzyme was subsequently found in mammalian cells and termed VHR for VH1-related (66). The majority of the members of the dual-specificity phosphatase group specifically dephosphorylate PThr and PTyr in one or several kinases that belong to the MAP kinase superfamily (reviewed in 67-69). Interestingly, there are currently 15 known dual-specificity phosphatases (often collectively termed MKPs), but only 10 known MAP kinases (Erk1, Erk2, Erk3, Erk5, Erk7, Jnk1, Jnk2, p38alpha, p38beta, and p38gamma). One reason for this excess of phosphatases might be that some of them have restricted expression profiles, while the MAP kinases are expressed in all tissues and cell types. Thus, only a few MKPs might be present in any one cell. The structure of these enzymes is presented in figure 2.

Finally, a number of much more distantly related proteins have been identified, which may be specific for PTyr, but in some cases are dual-specific or have an unclear specificity in intact cells. These enzymes share the $C(X)_5R$ motif with the classical PTPases, and include the cell cycle regulators CDC25 (70) and KAP (71), and the low Mr PTPases LMPTP (72), PRL-1 (73), PRL-2 (74), PRL-3 (74). Finally, two enzymes that have the PTPase signature sequence, PTEN (75,76) and CEL-1 (77), were recently discovered to be specific for non-protein substrates: PTEN dephosphorylates inositol phospholipids (78) and the *C. elegans* protein CEL-1 dephosphorylates RNA as part of the mRNA capping process (77). Thus, the "PTPase signature sequence" is not unique to enzymes hydrolyzing phosphoesters in protein substrates. Rather it seems that the PTPases have evolved from ancestral cysteine-based hydrolases specific for many other types of substrates. These primordial enzymes apparently provided a starting point for the evolution of PTPases in parallel with the appearance of dual-specific protein kinases (e.g. Mek) and PTKs during the transition from unicellular to multicellular organisms some 570-600 million years ago.

Lymphocytes express many, but not all, of the known PTPases (table 1). The leukocyte common antigen, CD45, seems to be the predominant transmembrane PTPase on most leukocytes, and it is expressed at very high levels in lymphocytes. Very little is known about other transmembrane PTPases in lymphocytes. For this reason, we have decided to bypass them in this review. Table 1 contains all currently known intracellular PTPases, including their numerous synonyms.

2.2. The targets and specificity of PTPases in lymphocyte activation

Many molecules that regulate lymphocyte activation become transiently phosphorylated on tyrosine following receptor ligation. As a general rule, these molecules are phosphorylated on multiple sites with each site potentially having its own specific impact on the function of the molecule. Each site may be phosphorylated by a different PTK (43,131), even if target sites for

Table 1. Mammalian cytoplasmic PTPases included in this review

PHOSPHATASE (SYNONYMS)	CHR. LOC.	PROTEIN Mr	IN T CELLS	REF[a)]
I. CLASSICAL PTPases				
HePTP group				
HePTP (LCPTP)	1q32.1	38-40 kDa	Yes	79,80
STEP	11p15.2-p15.1	20[b)], 38[b)], 46, 61 kDa	No	81
PCPTP (=PTPBR7, PC12-PTP1)		42, 65 kDa	No	82, 83, 84
TypPTP		40, 45 kDa	No	85
PTP1B group				
PTP1B	20q13.1-13.2	50 kDa	No	59
TCPTP (=MPTP, PTP-S)	18p11.2-11.3	45, 48 kDa	Yes	86
SH2 domain-containing group				
SHP1 (=PTP1C, SH-PTP1, HCP, PTPN6)	12p12-13	67 kDa	Yes	87-90
SHP2 (=SH-PTP2, Syp, PTP1D, PTP2C, SH-PTP3, PTPN11)	12q24.1	70 kDa	Yes	91-94
PEST-containing group				
PEP		110 kDa	Yes	87
PTP-PEST (=PTP-P19, PTPG1)	7q11.23	89/97 kDa	Yes	95-97
PTP-HSCF		50 kDa	No	98-102
Lipid-binding homology-containing group				
PTP-MEG2		68 kDa	Yes	103
ERM domain-containing group				
PTPH1		104 kDa	Yes	104
PTP-MEG1		106 kDa	Yes	105
PTPD1 (=PTP2E, PTP-RL10)		130 kDa	No	106-108
PTPD2		unknown	No	106
BA14		290 kDa	No	109
PTP-BAS (=FAP-1, PTP1E, RIP, PTPL1, PTP-BL, PTPN13)	4q21.3	275 kDa	Yes	110-113
PTP36 (=PEZ)	1q32.2-41	130 kDa	Yes	114,115
II. DUAL-SPECIFIC PHOSPHATASES				
Large MKPs				
Pac-1 (=DUSP2)	2q11.2-q11	32 kDa	Yes	116
MKP-1 (=CL100, 3CH134, erp,hVH1)	5q34-35	39 kDa	Yes	117-119
MKP-2 (=TYP1, hVH2, DUSP4)	8p11-p12	43 kDa	Yes	120,121
MKP-3 (=Pyst1, rVH6, DUSP6)	12q22-q23	42 kDa	No	122,123
MKP-4	Xq28	42 kDa	?	124
MKP-5	1q32 or 1q41	53 kDa		458,459
MKP-7	12p12	73 kDa		461
B23 (=hVH3)	10q25	44 kDa	?	127
hVH4	10q11	?	?	128
M3/6 (=hVH5, HB5)	11p15.5	69 kDa	?	129,130
MKP-X (=Pyst2, B59, DUSP7)	3p21	39 kDa	Yes	122,125,126
Small MKPs				
VHR	17q21	21 kDa	Yes	66
VHX	17q21	20 kDa	Yes	492
MKP-6	17q21	22 kDa	Yes	460
TMDP	17q21	23 kDa	No	456
DSPs with Zinc finger				
hYVH1	1q21-q22	38 kDa	Yes	457
Cell cycle regulators				
CDC25A	3p21	52-72kDa	Yes	70
CDC25B	20p13	52-72kDa	Yes	70
CDC25C		52-72kDa	Yes	70
KAP		24 kDa	?	71
Tumor suppressor				
PTEN (=MMAC1, TEP1)	10q23.3	55 - 60 kDa	Yes	75,76
PTEN2	8	~77 kDa	No	644
III. SMALL, UNRELATED ENZYMES				
LMPTP(low MrPTP,AcP,BHPTP)	2p25	18 kDa	Yes	72
PRL-1	6q12	20 kDa	Yes	73
OV-1 (PRL-2, PTP4A)	17q12-21	20 kDa	Yes	74
PRL-3		20 kDa	No	74

[a)] Original cloning papers [b)] Isoforms devoid of PTPase domain

individual PTKs sometimes are clustered. Naturally, all these proteins must also be substrates for cellular PTPases. It remains unknown, however, how many PTPases are required to dephosphorylate all these substrates. Are PTPases very specific for their substrates? How many substrates does each PTPase have? The case of the MAP kinase-specific phosphatases suggests that the number of phosphatases may even exceed the number of their targets.

Recent findings indicate that many PTPases display a high degree of substrate specificity. As in the case of the PTKs, PTPases are often (but not always) remarkably selective when tested *in vitro* towards phosphorylated peptides and proteins, and it is likely that they are even more selective in intact cells. Some PTPases seem to prefer PTyr residues preceded and followed by specific amino acid residues (132,133). For example, PTP1B strongly prefers acidic residues N-terminal of the target PTyr and a methionine or glycine immediately C-terminal of it (132). In intact cells, this intrinsic substrate specificity is combined with a selective targeting of the enzyme to specific subcellular compartments or to multisubunit protein complexes by the presence of protein-protein interaction domains or "zip codes" (134). Examples include the binding of SHP1 and SHP2 via their SH2 domains to certain tyrosine phosphorylated sites on other proteins, the association of the non-catalytic C-termini of TCPTP with substrate proteins or regulators, the binding of proteins with SH3 domains to the proline-rich regions of PTP-PEST and PEP, and the potential association of some PTPases with transmembrane proteins or cytoskeletal elements via ERM and PDZ domains. Superimposed on these two mechanisms to ensure selectivity is a third mechanism that consists of upregulation of PTPase activity by interaction with substrate, which is often achieved by similar means as the subcellular targeting. These will all be discussed in detail below.

An important group of PTPase substrates are the PTKs involved in lymphocyte signal transduction. With the probable exception of Csk, these enzymes are all negatively and positively regulated by phosphorylation of multiple tyrosine residues (5-9,43,44,131,135-138). The CD45 PTPase is known to positively regulate the Src family PTKs by dephosphorylating their C-terminal negative regulatory site (discussed below). Other PTPases may also act on this site (139). Several other PTPases are very likely to participate in the regulation of the PTKs, particularly by dephosphorylating the sites that CD45 does not act on. We have observed that the autophosphorylation site of Lck, Tyr-394, and the site in the SH2 domain, Tyr-192, both become rapidly phosphorylated in cells treated with PTPase inhibitors (131,140), suggesting that they are normally efficiently dephosphorylated by one or several PTPases. Likewise, both Syk (141) and Zap (142) become hyperphosphorylated within seconds after the addition of pervanadate. This response is also seen in CD45-negative cells (our unpublished observation). Taken together, these observations suggest that these PTKs are under very efficient control by multiple PTPases.

A large number of substrates for the PTKs have been identified, although it remains largely unknown which PTK phosphorylates these substrates under physiological conditions. It is even less clear which PTPases that dephosphorylate them. These signaling molecules include upstream components of signaling pathways and in many instances their phosphorylation has been shown to switch on the pathway. For example, phosphorylation of phospholipase Cgamma1 at Tyr-783 leads to its activation (143-147) and subsequent hydrolysis of membrane inositol phospholipids, calcium mobilization mediated by inositol trisphosphate and activation of protein kinase C through diacylglycerol formation (148). Another mechanism by which tyrosine phosphorylation regulates signaling events is by inducing physical association of proteins. For example, phosphorylation of the LAT (149) and Shc (150) proteins, causes them to bind the Grb2 adapter molecule, which, in turn, associates with Sos, a guanine nucleotide exchange factor for the small membrane-associated G protein Ras (151,152). Activated GTP-bound Ras then recruits the cytosolic inactive form of the Ser/Thr protein kinase c-Raf, causing its membrane translocation and activation (153,154). Raf, in turn, activates a cascade of Ser/Thr kinases, including Mek (155,156), which activates the mitogen-activated protein (MAP) kinases Erk1 and, in T cells particularly, Erk2 (157,158). Erk1 and 2 then phosphorylate additional kinases and the cascade culminates in the phosphorylation of transcription factors and cell cycle regulators (159,160). An important downstream target of Erk is the Elk-1 transcription factor, which participates in the transcriptional control of the c-*fos* protooncogene. Erk is known to be important in many events of lymphocyte physiology, such as development (161), cytokine production (162), proliferation (163) and anergy (164).

A parallel, but distinct, kinase cascade is initiated by tyrosine phosphorylation of Vav (165), an important (166,167), 95-kDa multidomain protein, which is a guanine nucleotide exchange factor for the small G protein Rac (168). GTP-bound Rac subsequently activates one or several protein kinases, which in turn activate Mkk4 (also called Sek), a dual-specificity protein kinase, which specifically activates the MAP kinases Jnk1 and Jnk2 (169,170). These two kinases are often referred to as stress-activated protein (SAP) kinases as this pathway is particularly efficiently switched on by stimuli such as heat shock, UV light, pro-inflammatory cytokines, etc. A main target for Jnk1 and 2 is the c-Jun protooncogene product (169), which together with the Fos proteins form the AP-1 dimer, an important transcription factor that cooperates with NFAT, NF-kappaB and Oct proteins in activation of cytokine genes in activated lymphocytes (171-174). A third kinase cascade includes Mkk3 and 6, which activate the p38 MAP kinase (175). There is at least one more, poorly understood, kinase cascade that leads to activation of the 80-kDa Erk5 MAP kinase.

Other cellular proteins that become tyrosine phosphorylated in triggered lymphocytes include the protooncogene product c-Cbl (176), a 120-kDa ubiquitin ligase with multiple potential tyrosine phosphorylation sites and proline-rich regions for binding of SH2 and SH3 domains, *e.g.* of phosphatidylinositol 3-kinase p85, Grb2

(177), and Zap-70 (178). The 76-kDa SLP-76 (179), which also has an SH2 domain, associates with the SH3 and SH2 domains of Vav and the SH3 domains of Grb2 (179). It was recently shown that SLP-76 is important in T cell development (180) and in TCR signaling for coupling the receptor to phospholipase Cgamma1 (181). The 36-38-kDa LAT protein (149) is also somehow involved in linking the TCR to phospholipase Cgamma1 (182), and it may serve as the main mechanism for tyrosine phosphorylation-dependent recruitment of phosphatidylinositol 3-kinase to the plasma membrane (183,184).

Finally, an interesting group of PTPase substrates are the PTPases themselves. Several PTPases have been reported to be phosphorylated on tyrosine residues (11-20). However, the significance of this modification is in most cases still unclear. Often the stoichiometry is very low and PTyr is only detected in the PTPase after treatment of the cells with potent PTPase inhibitors, such as phenylarsine oxide or pervanadate. On the other hand, in some cases a catalytically inactive mutant of the PTPase becomes much more phosphorylated than the active enzyme, suggesting that it normally is able to autodephosphorylate. This would explain why strong pharmacological inhibition is required to preserve the phosphorylation through the immunoprecipitation procedure. It could also be argued that even a very low stoichiometry phosphorylation could be highly significant in intact cells, for example if the phosphorylated form specifically interacts with other proteins. The relevance of tyrosine phosphorylation of PTPases can only be tested by mapping the site(s), changing these tyrosine residues to phenylalanines by site-directed mutagenesis, and using these mutants in functional experiments.

3. CLASSICAL PTPases

3.1. CD45 - a positive regulator of T cell activation

The first PTPase whose function in TCR-initiated T cell activation was elucidated is the transmembrane receptor-like CD45 PTPase, which is abundantly expressed on the surface of all nucleated hematopoietic cells (185,186). The enzyme has a long and slender extracellular domain, which is variable due to alternative splicing of exons 4, 5, and 6 during processing of the primary transcript. As a result the mature mRNA encodes a protein that contains all, some, or none of the sequences encoded by these three exons. The smallest CD45 isoform, termed CD45-R0, lacks these sequences and has a relative molecular weight of 180 kDa, while the largest, CD45-RABC, contains all exons and has a Mr of 220-240 kDa. The extracellular domain of CD45 is also heavily glycosylated being rich in sialic acid and galactose amine (187). These carbohydrates make CD45 a good ligand for some lectins, including animal galectins (188). Different leukocyte populations and subtypes have different expression patterns of CD45 isoforms, each individual cell usually expressing several isoforms. It seems likely that the different isoforms have somewhat different physiological functions, perhaps through differences in interaction with other surface proteins resulting in enhanced or reduced juxtaposition with other signaling molecules (e.g. CD4-Lck). Our understanding of the alternative splicing of CD45 and its role in lymphocyte physiology is still rudimentary. Nevertheless, it seems that much of the initial enthusiasm sparked by the discovery that it is a PTPase (60), now has dissipated and that only a few brave laboratories still focus on this molecule and its role in leukocyte physiology. Consequently, our understanding of CD45 has progressed relatively little in the last few years. As recent reviews (e.g. 186) contain essentially all current knowledge, we will summarize the state-of-the-art quite briefly.

T lymphocytes that lack CD45 fail to respond to stimulation by antigen or mitogenic antibodies (189). Responsiveness is restored upon reexpression of wild-type CD45 (190), or by expression of chimeric molecules containing the intracellular domain of CD45 fused to transmembrane and extracellular parts from heterologous proteins (191,192) or by the intracellular domain of CD45 alone (193) targeted to the plasma membrane by addition of the myristoylation signal contained within the first N-terminal amino acids of c-Src, the "SH4 domain" (5). Under physiological conditions, however, it is likely that the alternatively spliced extracellular domain of CD45 also plays an important role (194), perhaps by regulating the interaction with other surface proteins. The requirement for CD45 in T cell activation seems to reside at a very early stage in the signal transduction cascade, since, in the absence of CD45, the rapid receptor-triggered tyrosine phosphorylation of cellular proteins is severely reduced (195), resulting in impaired activation of phospholipase C (196) and disturbed calcium homeostasis (197). Experiments with B lymphocytes and other leukocytes have largely confirmed the central role of CD45 in signaling through receptors that primarily use Src family PTKs for signal transmission. This effect also manifests as a failure in thymic development in mice with CD45-negative thymocytes lacking exon 6 of the CD45 gene (198). In addition, it was recently reported that the Jak kinases are also physiological substrates for CD45 (199). The dephosphorylation of Jaks leads to their inactivation and diminished signaling by cytokine receptors that use the Jak-Stat pathways. Thus, CD45 plays a positive role in the initial activation of lymphocytes, but a negative role in later signaling required for proliferation and activation of effector functions.

It seems that the main mechanism by which CD45 influences TCR-induced tyrosine phosphorylation is by positively regulating the Src-family PTKs Lck and Fyn (5-10, 200-203) in T cells, and other members of the Src family in other leukocytes. Dephosphorylation of these kinases occurs at their C-terminal negative regulatory site (reviewed in 5), and correlates with responsiveness of the T cells to TCR stimulation (202). Consequently, we have hypothesized (9) that the main physiological role of CD45 is to counteract the suppressive effect of the Csk kinase, which rephosphorylates the C-terminal tail of Src family PTKs (reviewed in 5), and thereby to keep a sufficiently large fraction of the cellular pool of Src family PTKs active and able to participate in signal transmission. This function of CD45 seems to be constitutive and not significantly

modified by receptor ligation. On the other hand, there is evidence that CD45 does not treat all Src family members equally. In T cells, for example, CD45 does not seem to have the same affinity for Fyn as for Lck. Although some skewing may arise from differences in expression levels or subcellular location, there is data to support the notion that the N-terminus of Lck allows the kinase to interact with CD45 better that other Src-like kinases (204). A physical association between Lck and CD45 can be visualized by co-capping and is strong enough for some co-immunoprecipitation.

The expression of CD45 has also been found to affect the phosphorylation state of Zap (142) and Syk (our unpublished observation) in one pair of $CD45^+$ and $CD45^-$ cells, the murine YAC-1 T lymphoma. A physical association of Zap with CD45 was seen by co-immunoprecipitation and by co-capping experiments and Zap was constitutively tyrosine phosphorylated in the $CD45^-$ YAC-1.N1 clone (142). Although a direct action of CD45 on Zap or Syk has not been excluded, it appears more likely that the effects of CD45 on Syk and Zap are indirect. There is evidence that TCR-zeta is a physiological substrate for CD45 (205). Since dephosphorylation of TCR-zeta would prevent the association of Zap and its subsequent activation by Lck, it seems that CD45 can have a negative influence on signaling, which is opposite to its positive effects of Lck and Fyn. This may explain the contradictory results obtained with anti-CD45 mAbs, which can be either stimulatory or inhibitory for T cell activation. It may also be important to note that results obtained in $CD45^-$ variants of several transformed T cell lines are partly contradictory. For example, while Lck is hyperphosphorylated only at Tyr-505 and has a low catalytic activity in most T cells lacking CD45, it also becomes hyperphosphorylated at the positive regulatory site, Tyr-394, and is catalytically activated in the YAC1.N1 (10). Although this effect is reversed by re-expression of CD45 (193), it is clear that CD45 is not the main PTPase responsible for Tyr-394 dephosphorylation as the authors propose. In other T cells lacking CD45, Tyr-394 of Lck is not phosphorylated, but phosphate does accumulate at this site if the cells are treated with a PTPase inhibitor (140). The YAC-1 cell line might be deficient in some other PTPase. PTPase inhibitors also induce hyperphosphorylation of Syk and Zap in T cells lacking CD45, suggesting that a PTPase distinct from CD45 dephosphorylates these PTKs as well.

The physical size of CD45 has puzzled researchers. The extracellular domain of CD45 forms a long rod, which exceeds the size of the TCR and its co-receptors several fold, and thereby would prevent interaction of the TCR with MHC molecules on the surface of another cell. There is recent insight into the mechanics of T cell contact with antigen-presenting cells that overcomes this dilemma. By immunofluorescence staining and computer-enhanced confocal microscopy, A. Kupfer's laboratory has found that most of CD45 leaves the small area that forms the contact with the antigen-presenting cell (206,207). At the same time, the TCR and its co-receptors accumulate in this area, as do Lck and other signaling molecules on the cytoplasmic face of the contact area. These results suggest that signaling from the TCR proceeds without being counteracted by the PTPase domains of CD45 as long as the contact with the antigen-presenting cell is maintained. As CD45 might be the PTPase that dephosphorylates the ITAMs of the TCR-zeta, the exclusion of CD45 would strongly promote the phosphorylation of these sites. On the other hand, the absence of CD45 in the contact area will also prevent dephosphorylation of the negative regulatory sites in Lck and Fyn, causing their inactivation. We have measured the turnover of phosphate at this site of these PTKs (140) and found it to be relatively slow. Thus, the activity of Lck and Fyn molecules that are out of CD45's reach in the contact area will remain active for sufficiently long periods of time. This new findings underscore the importance of studying PTPases not only on a biochemical level, but also as components in a highly organized, three-dimensional context within living cells.

3.2. HePTP - a cytosolic leukocyte-specific PTPase targeted to MAP kinases

The hematopoietic protein tyrosine phosphatase (HePTP), also known as LCPTP, was originally cloned from human T lymphocytes (79,80). The gene is expressed exclusively in thymus, spleen, in all leukocyte types, and in most leukemic cell lines examined, including the Jurkat T leukemia cells (our observation), HL-60 (promyelocytic leukemia), NALM-6 (pre-B cell line), Tall-1 (T cell leukemia cell line), PEER (T cell leukemia line) and RPM18226 (myeloma cell line). HePTP is not detected in non-hematopoietic tissues (79,80,208) or cell lines. HePTP belongs to a group of PTPases with currently 3 other members, STEP (for striatum-enriched phosphatase; ref. 81), PCPTP1 (82-84), and Typ (85). Interestingly, at least the two former come in many different isoforms due to alternative mRNA splicing (209-211) or translational initiation (82). The four genes of this family are all expressed in different tissues; STEP mainly in brain, particularly, striatum (81) and PCPTP1 in lung, heart and brain, particularly in cerebellum, but not in striatum (82), and Typ only in spermatocytes (85). Thus, hematopoietic cells express only HePTP and none of the other family members.

There is some controversy regarding the first N-terminal amino acids of HePTP. B. Zanke's group (79) cloned the cDNA for HePTP from human peripheral T lymphocytes and found the open reading frame to encode a protein of 339 amino acids (38 kDa). Simultaneously, Adachi's team (80) isolated a cDNA clone from a human T cell PEER cDNA library, which encoded a protein of 360 amino acids (40 kDa), which they termed LCPTP for Leukocyte PTP. The translation start codon was identified at different positions in the two cDNAs. As the result LCPTP was found to have 21 extra amino acid residues at the N-terminus, while nearly identical in the remainder of sequence to HePTP. The cDNA encoding the rat equivalent of the human HePTP/LCPTP from a rat basophilic leukemia mast cell cDNA library was recently isolated (212). The rat and human sequences share 92% identity at the amino acid level. The protein expressed in the mast

cells was of a size about 40kDa and agrees with LCPTP.

The exon/intron structure of the HePTP/LCPTP gene has been determined (208, 213). It is located on chromosome 1q32.1 and is organized into 11 exons. The open reading frame was determined to start in the middle of exon 2 and terminates in the exon 10. Exon 1 has not been identified to date. The exon/intron structure is very similar to that of the phosphatase domains of human CD45 (214). Particularly, exons 4 through 10 encoding the conserved catalytic PTP domain are located at the same position in both HePTP and CD45 gene. We note in passing that chromosome 1q31-q32 contain genes of two other PTPases expressed exclusively in hematopoietic cells, LAR and CD45, which have 55% and 53% homology, respectively. In contrast to both of these PTPases, HePTP contains only a single PTPase domain, which occupies the C-terminal 3/4 of the enzyme and is preceded by non-catalytic N-terminus (figure 1).

The lack of putative transmembrane sequences or recognizable signal motifs in HePTP for subcellular targeting suggests that the enzyme might be soluble in the cytosol. Indeed, immunofluorescence microscopy indicates that HePTP is localized only to discrete globular compartments within the cytoplasm of mast cells and not in the nucleus or associated with the cell surface membrane (212). In T cells, the immunofluorescence is quite evenly distributed throughout the cytosol (our unpublished observation).

Experiments conducted the last few years have begun to shed some light on the biological function of HePTP. Ectopic expression of HePTP in NIH3T3 cells resulted in altered cell morphology, disorganized growth, anchorage independent colony formation and subtle differences in the pattern of tyrosine phosphoproteins compared to control cell lines (213). In hematopoietic cells, the first indication of a role of HePTP in cell proliferation or differentiation came from the finding that the HePTP gene is located on the long arm of chromosome 1, which is often found in extra copies (trisomy) in bone marrow cells from patients with myelodysplastic syndrome (215, 216), which is characterized by reduced hematopoiesis and increased risk of acute leukemia. Indeed, amplification and overexpression of HePTP has been reported in a case of myelogenous leukemia (213). Deletions of 1q32 have also been reported in non-Hodgkin lymphomas and chronic lymphoproliferative disorders (217). Together, these findings suggest that excess HePTP may correlate with reduced proliferation (in myelodysplasia) and loss of HePTP with increased cell proliferation and/or survival. A connection with proliferation is also supported by the finding that the HePTP gene is transcriptionally activated in T cells treated with interleukin-2 (180). Although mRNA levels also increased several fold upon stimulation of normal mouse lymphocytes with phytohemagglutinin, lipopolysaccharide, Concanavalin A or anti-CD3 (79), the HePTP protein was present in resting cells and its amount increased only moderately. HePTP has also been reported to become phosphorylated on tyrosine in RBL-2H3 mast cells stimulated through their Fc-epsilonRI (212).

It was shown that transient expression of HePTP in T cells causes a clear reduction in antigen receptor-induced transcriptional activation of a reporter gene driven by a NFAT/AP-1 element taken from the interleukin-2 gene promoter (218). In contrast, a catalytically inactive C270S mutant of HePTP had no effect, suggesting that the PTPase activity of HePTP was required for inhibition (218,219). The same results were observed for LCPTP (220). HePTP also reduced TCR-induced activation of the mitogen-activated protein (MAP) kinase Erk2, but not of the N-terminal c-Jun kinase (Jnk). Expression of wild-type LCPTP in 293T cells suppressed the phosphorylation of Erk2 by mutant MEK1, which was constitutively active regardless of upstream activation signals (220). HePTP also did not affect the activity or phosphorylation of MEK, the upstream activator of Erk. Furthermore, HePTP binds to Erk2 and p38 kinase in T cells (219, 220). Other investigators found that only Erk2 bound well to HePTP, while p38 bound weakly at low-salt conditions (221). The same study showed that Erk-HePTP binding depend on Erk tyrosine phosphorylation i.e. HePTP appears to be targeted to activated Erk (221). In the bound state HePTP dephosphorylates the critical phospho-tyrosine in activation loop of Erk2 and p38 (219). The region of HePTP responsible for the specific binding to Erk and p38 was mapped to the non-catalytic N-terminus of HePTP. This non-catalytic domain contains Kinase Interaction Motif (KIM) which was consistently found in other kinase binding PTPases, PTP-SL and STEP (222), PTP-ER (223), MKP-3 (224) and in the yeast Ptp3 (225). Deletion of a part of this non-catalytic domain of HePTP/LCPTP or substitutions R41A or R42A (in LCPTP) causes complete loss of ERK binding (218, 219). Moreover, the ERK mutant D321N does not form a complex with LCPTP (219). Upon formation of complex ERK may phosphorylate HePTP at T45 and S72 residues.

Clearly, much remains to be learned about the function of HePTP/LCPTP in intact cells, it appears that its main task is to control the Erk and p38 MAPK kinase signaling pathway. Whether HePTP/LCPTP only helps maintain these pathways in a resting state and tempers their activation, or if it regulates other aspects of MAP kinase signaling (e.g. subcellular location, timing, crosstalk, or downstream targeting) remains to be elucidated. It is also curious that HePTP/LCPTP will convert dually phosphorylated, active, Erk into an inactive, singly Thr-phosphorylated form that could potentially be activated by a PTK.

3.3. TCPTP - a PTPase of the endoplasmic reticulum

The T cell PTPase, TCPTP, is an intracellular enzyme with a single catalytic domain, which is located in its N-terminus (figure 1). Its cDNA was originally isolated in 1989 by Deborah Cool and co-workers from a human peripheral T-cell cDNA library (86), and it encodes a 48-kDa protein (86,226) with 65% sequence identity to the first cloned PTPase, PTP1B (59). In 1992, Mosinger and co-workers (227) described a cDNA encoding the mouse homologue of TCPTP (termed MPTP), which predicted a protein of 383 amino acids with a Mr of 45 kDa. The similarity between MPTP and human TCPTP is 88.8% at

the nucleic acid level and 93.2% at the amino acid. However, the human and murine open reading frames differ markedly at their 3' ends. This puzzle was solved by the realization that there is a splice donor site (AGGT) present in the human TCPTP cDNA at the position where the sequence diverges from the murine cDNA, suggesting the primary transcript undergoes an alternative splicing event that results in two isoforms differing in their C-termini. This has been confirmed. The larger protein is approximately 48 kDa and its C-terminus contains a hydrophobic and basic endoplasmic reticulum-retention signal, which is lacking in the smaller, 45-kDa isoform (227,228). Instead the smaller protein contains a nuclear localization signal, Arg-Lys-Arg-Lys-Arg, which immediately precedes the splice junction (229). Accordingly, the 45-kDa isoform is nuclear. The rat homologue of TCPTP (termed PTP-S for protein tyrosine phosphatase of spleen) has also been cloned (230) and it is 95% identical to the human protein within the catalytic domain. There is also evidence for alternative splicing in the rat, perhaps giving rise to more than two isoforms (231).

Although TCPTP was isolated from T cells (86) and from spleen (227,230), it is not restricted to lymphoid cells, but is found in a variety of cell types and tissues. The expression is low in embryonic stem cells and increases during later stages of mouse development (227). Highest expression of the major RNA transcript of 1.9kb was observed in the ovaries, testes, thymus and kidneys. A second RNA transcript of ~1.3kb, was detected exclusively in the testes. The TCPTP gene was found to be located on chromosome 18p11.2-11.3 (232).

Mice with a $TCPTP^{null}$ mutation were recently generated in M. Tremblay's laboratory (233). The three genotypes (+/+, +/-, and -/-) showed the normal Mendelian 1:2:1 segregation at birth demonstrating that the absence of TCPTP was not lethal in utero. However, all $TCPTP^{-/-}$ mice failed to grow normally and died by 3-5 weeks of age, having enlarged spleens and lymph nodes. These mice also exhibited specific defects in their bone marrow, in B cell lymphopoiesis, and in their erythropoiesis, as well as impaired T and B cell functions. However, myeloid and macrophage development in the bone marrow and T cell development in the thymus were not significantly affected. Furthermore, bone marrow transplantation experiment showed that the lymphoid problems in $TCPTP^{-/-}$ animals were not due to defects in the hematopoietic stem cells, but rather to a stromal cell deficiencies. Although these findings do not exclude the possibility that TCPTP plays an important role in lymphocytes, they indicate that TCPTP is not an indispensable component of the lymphocyte development or activation machinery.

Despite the generation of knock-out mice, the physiological function and substrates of TCPTP remain poorly understood. The high degree of sequence similarity between TCPTP and PTP1B, with as high as 85% sequence similarity within the catalytic domain, predicted that they may have similar physiological functions and modes of regulation. This prediction has been partly fulfilled, but there are also important differences between the two proteins. Both enzymes are targeted to the endoplasmic reticulum through their carboxy-terminal segments (226,234-238). The C-termini also suppress the catalytic activity of the native enzymes (234), suggesting the existence of an intramolecular regulatory mechanism. Accordingly, truncation of the C-terminus of TCPTP results in a constitutively active cytosolic protein (234). Expression of such a 37-kDa C-terminally deleted form of TCPTP caused BHK cells to become multinucleated and it suppressed v-*fms*-induced transformation of rat-2 cells (239,240). However, correct subcellular localization is likely to be crucial for targeting TCPTP to its physiological substrates, and overexpression of wild-type TCPTP has little effect on the morphology or growth of cells.

Recently, a number of associated proteins and putative substrates for TCPTP have been described. Tiganis and co-workers (241) isolated three proteins, p97, p116 and p120, that interacted with the alternative C-termini of TCPTP. p120 interacted with the longer C-terminus, while p116 and the nuclear import factor p97 bound to the basic residues of the TCPTP bipartite nuclear localization signal, which targets the 45-kDa TCPTP isoform to the nucleus. These findings are consistent with the notion that the alternative C-terminal segments of the TCPTP isoforms target the enzyme to defined intracellular locations through specific interactions with regulatory proteins.

Lammers and co-workers (242) reported that both PTP1B and the 48-kDa isoform of TCPTP preferentially dephosphorylated the precursor forms of several receptor tyrosine kinases during their synthesis in the endoplasmic reticulum. This feature was not shared by two other intracellular PTPases, PTPH1 and SHP-1. Substrates included the epidermal growth factor receptor and the adapter protein Shc (243). It appears that PTP1B and TCPTP counteract the autophosphorylation of receptor tyrosine kinases during their synthesis and posttranslational processing in the endoplasmic reticulum. We have found that expression of TCPTP in T cells, even at 10-100 fold above endogenous levels, has no effects on TCR signaling as measured by many different parameters from early tyrosine phosphorylation events to the transcriptional activation of the interleukin-2 gene. However, TCPTP does reduce the basal tyrosine phosphorylation of several PTKs if they, too, are overexpressed at sufficiently high levels, presumably being abundant also in the endoplasmic reticulum. In agreement with the notion that the 48-kDa TCPTP has a localized housekeeping function, we have found that this enzyme is quite promiscuous *in vitro* compared to other PTPases (unpublished observation).

The role of the smaller form of TCPTP is unclear. A substrate-trapping mutant (D182A) of the 45-kDa TCPTP isoform transiently overexpressed in COS cells underwent a change in localization in response to epidermal growth factor, exiting the nucleus and accumulating in the cytoplasm (241). At the same time, it bound PTyr-containing proteins of Mr 50, 57, 64, and 180 kDa. Of these, the 57- and 180-kDa proteins were identified as Shc and the epidermal growth factor receptor, respectively. No

effects were seen on epidermal growth factor-induced MAP kinase activation, but the association of Shc with Grb2 was reduced. Whether the endogenous 45-kDa TCPTP remains in the nucleus at all times or cycles to the cytoplasm, remains to be studied. A number of nuclear proteins contain PTyr, including MAP kinases, Stat and other transcription factors and the Abl kinase and its substrates. The 45-kDa form of TCPTP may act on any of these, and could thereby have an important regulatory role in cell growth, differentiation or apoptosis.

3.4. SHP1 - a negative regulator of signaling

The 100-amino acid residue Src Homology 2 (SH2) domain is a hemispherical protein-protein interaction domain that specifically binds to PTyr-containing peptide sequences in other cellular proteins. Two PTPases are currently known that have SH2 domains, namely SHP1 (previously PTP1C, SHP, HCP, or SH-PTP1) and SHP2 (also known as PTP1D, PTP2C, SH-PTP2, or Syp). These two PTPases are similar in overall structure; both have two SH2 domains in their N-terminus and a catalytic PTPase domain in their C-terminus followed by a short C-terminal tail with two tyrosine phosphorylation sites. SHP1 is expressed at highest levels in cells of hematopoietic origin, while SHP2 is ubiquitous. Lymphocytes contain easily detectable levels of both. An alternative splicing of a 39-amino acid fragment in the C-terminal SH2 domain of SHP1 (244-246) creates some heterogeneity in the apparent molecular size of SHP1. There are also two naturally occurring point-mutations in the *shp1* gene in the two mouse strains *motheaten* (*me*) and *viable motheaten* (me^v) (247), in the former leading to premature termination of the protein, and in the latter strain causing the expression of functionally deficient SHP1 protein (244-247). These two mouse strains have been instrumental for the elucidation of the function and importance of SHP1 and have thereby also sparked interest in other PTPases as well. The vast majority of the numerous papers published on SHP1 indicate that SHP1 acts as an important negative regulator of many signaling systems in hematopoietic cells.

The posttranslational regulation of the catalytic activity of the SHP1 and SHP2 proteins is at least partly clear. The role of the two SH2 domains seems to be twofold: regulation of the catalytic activity of the enzyme and physical localization of the protein to its physiological sites of action. The first task is accomplished by a physical association of the N-terminal SH2 domain with the catalytic domain resulting in a strong suppression of the phosphatase domain (248,249). This interaction, which occurs in a PTyr-independent manner, is disrupted by binding of a PTyr-containing peptide ligand to the SH2 domains. As a consequence of SH2 domain engagement, the catalytic domain is liberated and activated up to 100-fold (248,250). As predicted by this intramolecular suppression model, investigators have found that removal of the SH2 domains increases the enzymatic activity (248,250-254). The second SH2 domain does not seem to participate in regulation of catalytic activity and only serves as a recruiting domain (248,249). Its importance lies in its participation in the synergistic binding of SHP1 to doubly phosphorylated ligands (255,256). Thus, the ligand specificity of the SH2 domains of SHP1 are largely responsible for juxtaposing SHP1 to certain phosphorylated cellular proteins that are, or associate with, the physiological substrates for the PTPase. At the same time, SHP1 is activated at the site of binding. On the other hand, studies where the tandem SH2 domains were exchanged between SHP1 and SHP2 indicated that substantial specificity also resides in the catalytic domain (257,258).

The ligand specificity of the SH2 domains of SHP1 was determined. The preferred sequence is generally referred to as the immunoreceptor tyrosine-based inhibition motif (ITIM), Val/Ile-Xaa-PTyr-Xaa-Xaa-Leu/Val (where Xaa represents any amino acid) (259-261). This motif is found in many different proteins, many of which are transmembrane receptors for inhibitory ligands (table 2).

Another potential mode of regulation is the phosphorylation of SHP1 at Tyr-538 (Tyr-536 in mouse SHP1) upon various extracellular stimuli (283-284), and also at Tyr-566 (Tyr-564 in mouse SHP1) in the Lck-overexpressing thymoma cell line LSTRA and in T cells (285). In vitro, Lck readily phosphorylates both these sites (our unpublished observation). Phosphorylation of these tyrosines does not seem to affect the enzymatic activity of SHP1, but both are in an optimal sequence for binding to the SH2 domain of the Grb2 adapter protein (286). Indeed, association of SHP1 with Grb2 has been reported in P815 mastocytoma cells and in bone marrow derived macrophages but not in T cells (287,288).

3. 4.1. Role of SHP1 in B cells

B lymphocyte activation is accomplished by binding of soluble multivalent antigen to the B cell antigen receptor (BCR) (289-291), which consists of a transmembrane immunoglobulin D or M molecule, having two heavy chains and two light chains, and at least two invariant glycoproteins Ig-alpha (MB-1) and Ig-beta (B29). Recognition of antigen is mediated by the variable portion of the Ig chains, and must lead to crosslinking or oligomerization of BCRs to cause signals to be transmitted into the B cell. While the Ig heavy chains have very short cytoplasmic tails (the light chains are solely extracellular), the Ig-alpha and Ig-beta polypeptides have long intracellular tails and are thought to be mainly responsible for signal transmission. A number of other transmembrane glycoproteins also participate as co-receptors, accessory molecules, or alternative routes of activation. These include CD19, TAPA-1, Leu13, the CR2 complement receptor (CD21) and CD40.

B cell activation is inhibited by co-ligation of the BCR with the type IIB receptor for the Fc portion of immunoglobulin G, Fc-gammaRIIB (259,260). Under physiological conditions, such co-ligation and juxtaposition of the two receptors only occurs when there are circulating antigen-antibody complexes. Under these circumstances, there is less need for more antibodies and, accordingly, B cell activation will be suppressed. The molecular mechanisms of this inhibition of BCR signaling by a juxtaposed Fc-gammaRIIB consists of the phosphorylation of Fc-gammaRIIB on tyrosine within its cytoplasmic ITIM sequence (260), followed by recruitment of SHP1 and

Table 2. ITIM containing receptors

RECEPTOR	LIGAND	EXPRESSION PATTERN	REFERENCE
I. Type I transmembrane molecules with Ig-like domains			
Fc-gamma-RIIB (human and mouse)	Fc of IgG	many lymphoid/myeloid cells	262
CD22 (human and mouse)		B cells	263,264
p58 (KIR, human)	MHC	NK cells, small subset of T cells	265,266
p70 (KIR, human)	MHC	NK cells, small subset of T cells	267,268
LAIR-1 (human)	?	NK cells, B cells, T cells, monocytes	269
LIR-1 (human)	?	B cells, monocytes	270
ILT-2 (human)	?	many leukocytes	271,272
ILT-3 (human)	?	myeloid cells, antigen presenting cells	273
ILT-4 (human)	?	myelomonocytic cells	272
ILT-5 (human)	?	myelomonocytic cells	272
gp49B1 (mouse)	?	mast cells, basophils, NK cells	274,275
p91/PIR-B (mouse)	?	B cells, myeloid lineage cells	276
MAFA	?	mast cells	277
II. C-type lectin group			
CD72 (human and mouse)	carbohydrate	B cells	278
NKG2A/B (human)	MHC	NK cells, small subset of T cells	279,280
Ly-49 (mouse)	MHC	NK cells, small subset of T cells	281,282

another SH2 domain-containing molecule, SHIP, which is a inositol polyphosphate 5-phosphatase (260). Inhibition of BCR signaling then occurs through dephosphorylation of protein substrates by SHP1 and inositol-containing phospholipids and soluble inositol polyphosphate second messengers by SHIP. The exact targets for SHP1 in this system are not entirely clear. It seems that dephosphorylation of CD19 (259,292) is important, and the finding that signaling by Fc-gammaRIIB was deficient in *me/me* mice, confirms that SHP1 plays an important role (259,293). In contrast, two reports suggested that SHIP, but not SHP1, is required for Fc-gammaRIIB-mediated inhibitory signaling in B cells (294,295).

SHP1 also associates with the ITIM-containing CD22 molecule (296), which also functions as an inhibitory signaling molecule in B cells. Results obtained with mice deficient in the B cell-specific Src family PTK Lyn, indicate that this kinase is required for the tyrosine phosphorylation of CD22, its association with SHP1, and the suppressive function of CD22 on BCR signaling (297). Additional ITIM-containing receptors, such as paired Ig-like receptor B (PIR-B), have been shown to associate with SHP1, SHP2, and SHIP *in vitro*, but only with SHP1 at detectable levels *in vivo* (298). When a chimera between the extracellular domain of Fc-gammaRIIB and the cytoplasmic domain of PIR-B was transfected into a Fc-gammaRIIB-negative mouse B cell lymphoma and co-crosslinked to the BCR, the intracellular free calcium release and NFAT activation was inhibited compared to stimulation by BCR crosslinking alone (299). The tyrosine phosphorylated chimera was able to bind SHP1 and SHP2, but not SHIP, and mutational studies showed that Tyr-771 and to some extent also Tyr-801 in the cytoplasmic domain of PIR-B were essential for maintaining the inhibitory capabilities of the chimera (299).

3.4.2. The function of SHP1 in Natural Killer Cells

Natural Killer (NK) cells are large granular lymphocytes that lack classical T or B cell antigen receptors and that express unique lineage-specific receptors, including CD56, CD16 and a number of killer cell inhibitory receptors (KIRs). The role of NK cells is to recognize defective host cells, e.g. tumor cells or virus-infected cells, and lyse them (300). Recognition is mediated by the KIRs (260,261,301), which bind to specific MHC class I molecules and suppress the innate killer instinct of the NK cell. Conversely, in the absence of normal class I molecules (e.g. if these molecules are missing, altered or foreign), the suppression does not occur and the NK cell destroys the target cell. NK cells also recognize antibody-coated target cells via their CD16 molecule, which is a transmembrane splice form of the type III Fc receptor for IgG (Fc-gammaRIIIA) (302-304). This 50-70 kDa glycoprotein has only 25 cytoplasmic residues, but associates with a homodimer of the zeta-chain (of the TCR) (305-307), or a heterodimer consisting of the TCR-zeta and the gamma chain of the high affinity Fc receptor for IgE (Fc-epsilonRI) (308). CD16 transmits a positive signal that induces killing of the target cells, termed antibody-dependent cytotoxicity (ADCC).

SHP1 is thought to be the operative signaling molecule in the function of the KIRs (294,295,309) and of the functionally, but not structurally, related NKG2A and B receptors (310). The cytoplasmic domain of the KIRs contain one or two ITIM sequences. SHP1 was reported to associate with the human p58KIR in a NK cell line, and introduction of an inactive SHP1 mutant in the same cell line reduced the inhibitory function of p58 in target cell lysis (309). The involvement of SHP1, but not SHIP, in KIR-mediated inhibition of target cell lysis has been supported by other investigations (294,295).

Subsets of T and NK lymphocytes express the CD94-NKG2A/B heterodimer, which recognizes the MHC class I molecule. Anti-CD16 induced specific cell lysis by a interleukin-2 dependent NK cell line was inhibited when

anti-NKG2A or anti-CD94 was crosslinked at the same time, and the same pattern was observed with anti-CD3-induced specific cell lysis by a melanoma specific cytotoxic T lymphocyte (CTL) clone (310). NKG2A contains two intracytoplasmic ITIM like sequences and when tyrosine phosphorylated, both of them can associate with the SH2 domains of SHP1 and 2 *in vitro* (but not with SHIP), suggesting that SHP1/2 are involved in the CD94/NKG2A mediated inhibition of both antigen induced T cell response and antibody induced NK cell cytotoxicity (310).

3.4.3. SHP1 in T cell signaling.

In contrast to the well established role of SHP1 in B lymphocytes and NK cells, it is less clear how important SHP1 is for T cell development and function. This is partly due to the absence of well-studied ITIM-containing inhibitory receptors on T cells. The recent cloned LAIR-1 might be such a molecule as it is expressed in most T cells (270), but its function is only now beginning to be investigated.

Studies with motheaten mice suggest that SHP1 may play a negative role in the T cell receptor (TCR) signaling. Thymocytes from these mice show enhanced and prolonged tyrosine phosphorylation of TCR-zeta and CD3 as well as prolonged MAP kinase activation upon TCR stimulation (311). It was also reported (312) that thymocytes from *me/me* mice incorporated 3- to 5-fold more [^{3}H]-thymidine in response to TCR-stimulation than thymocytes from normal mice. The response to interleukin-2 response was unchanged (312). This paper also showed increased tyrosyl phosphorylation of several cellular substrates, correlating with increased kinase activity of the Src-family kinases Lck and Fyn, suggesting that SHP1 is involved in the inactivation of Lck and Fyn (312). Interestingly, the catalytic activity of c-Src in thymocytes from *me/me* mice was substantially lower than in normal thymocytes, but could be enhanced by *in vitro* exposure to SHP1 (313). Furthermore, SHP1 preferentially dephosphorylated c-Src isolated from Jurkat T cells at its inhibitory site of tyrosine phosphorylation, Tyr-527, indicating that SHP1 is involved in the activation of this kinase (313). This is apparently not true for other related kinases (e.g. Lck and Fyn), and it seems not to have any significant effect on T cell activation. In Jurkat T cells, expression of a chimera between the extracellular and transmembrane domain of the HLA-A2 molecule and SHP1 was unable to rescue TCR-mediated signal transduction in the CD45-deficient J45.01 variant of Jurkat (314). However, expression of the chimera in normal Jurkat cells diminished the inositol phosphate production as well as NFAT/AP-1 promotor activity, confirming that SHP1 can regulate the TCR response. Finally, the direct regulation of ZAP-70 by SHP-1 in T cells has also been suggested (315). Other investigations based on *in vitro* studies or transfection of heterologous cells also show that SHP1 has many potential substrates, e.g. co-expression of SHP1 with ZAP-70, Lck, and TCR-zeta chain in Sf21 insect cells show that SHP1 can dephosphorylate all of them *in vivo* (316).

In contrast to the finding that the response to interleukin-2 is unaltered in *motheaten* thymocytes, Migone and coworkers (317) showed that interleukin-2 induced association of SHP1 to the interleukin-2 receptor complex of T cells, and that SHP1 was able to decrease the tyrosine phosphorylation level of the 75-kDa beta subunit of the interleukin-2 receptor and the associated tyrosine kinases JAK1 and JAK3 (317). Interestingly, SHP1 expression is decreased or undetectable in many interleukin-2 independent HTLV-I transformed T cell lines that exhibit constitutive JAK/STAT activation, indicating that SHP1 downregulates the interleukin-2 induced signaling response in T cells (317).

3.4.4. Role of SHP1 in other hematopoietic receptor systems.

SHP1 also appears to play a negative regulatory role in signaling through the erythropoietin receptor (EpoR) (318,319), growth hormone receptor (320,321), the stem cell factor receptor, c-Kit (322-324), colony-stimulating factor-1 receptor, CSF-1R (288,325), the receptor for interleukin 3 (326,327), and the receptors for interferon alpha and beta (328,329). These receptors are either PTKs themselves (c-Kit and CSF-1R), or use Jak family PTKs for signaling. Generally, SHP1 binds directly to these receptors via its SH2 domains and subsequently dephosphorylates either the receptor, the associated PTKs, or their substrates. For example, in the case of the EpoR, receptor ligation induces recruitment of Jak2, which phosphorylates itself, the receptor, and other proteins, thereby initiating a signaling cascade that leads to growth and/or differentiation. Following phosphorylation of EpoR at Tyr-429, SHP1 binds to this site via its SH2 domains and subsequently dephosphorylates Jak2 leading to termination of the proliferative signals (318,319). SHP1 also binds to c-Kit via its SH2 domains, and observations made in *motheaten* mice suggest that SHP1 directly dephosphorylates and regulates c-Kit (327). The preferred binding site of SHP1 on c-Kit is Tyr-569, and Tyr-567 might contribute to this interaction (323). When Tyr-569 was mutated to phenylalanine and the construct transfected into Ba/F3 cells, the association was disrupted and the cells hyperproliferated in response to stem cell factor. Macrophages from *me/me* mice also proliferate more in response to CSF-1 than control cells and their CSF-1R is hyperphosphorylated, indicating that SHP1 also is a critical negative regulator of CSF-1R signaling (288). The negative feedback role of SHP1 in all these receptor systems probably explain the multiple and severe abnormalities in the development and function of the hematopoietic cells in the *motheaten* mice (244,245,247).

In conclusion, it is becoming clear that SHP1 is an important negative regulator of most hematopoietic-specific signaling systems. In contrast, expression of SHP1 in non-hematopoietic cells, does not affect, or even augments, signaling from receptors for epidermal growth factor, platelet-derived growth factor, insulin or interferon-gamma (330-333). Although the mechanisms for SHP1 recruitment have been elucidated in great detail, the exact substrates for SHP1 in most receptor systems remain unclear.

3.5. SHP-2 - a positive regulator of signaling?

Although SHP2 shares the molecular architecture of SHP1 and is highly homologous at the amino acid level,

it evidently has unique functions in cell signaling (334,335). Among the many functions described so far, most documented is the positive role of SHP-2 in mediating the activation of extracellular signal-regulated kinase (Erk) by growth factors and cytokines. However, it remains unclear how Shp-2 acts to promote signal relay from receptor PTKs to Erk. Experimental data from many different groups suggest that the catalytic activity of SHP-2 is required for this function in cells. This would suggest that a phosphorylation event needs to be reversed or prevented for a full-scale activation of Erk kinase by growth factors and cytokines.

3.5.1. Role of SHP-2 in mediating ITIM function

Compared to SHP-1, much less is known about the physiological function of SHP-2 in lymphocyte signaling. Previous biochemical data suggest that SHP-2 participates in signaling events downstream of receptors for antigens and IL-2, and SHP-2 physically interacts with a number of cell surface and cytoplasmic signaling proteins in lymphocytes (17,336,337). However, the physiological significance of these protein complexes is not clear. RAG-2 complementation assay with homozygous Shp-2 mutant embryonic stem cells demonstrated that a functional SHP-2 is positively required for normal lymphopoiesis (338). There were no mature T and B cell detected in chimeric animals derived from Shp-2-/- ES cells injected into RAG-2-/- blastocysts, This result clearly shows an essential role of SHP-2 in lymphopoiesis, a function distinct to SHP-1.

It was recently reported that a small transmembrane protein named SIT (for SHP-2-interacting transmembrane adapter protein) interacts with SHP-2 via its intracellular domain (339). SIT is a novel disulfide-linked dimer that contains ITIM motifs at the intracellular domain. The function of SIT in T cell signaling is not clear, it might act as a negative effector as overexpression of SIT in Jurkat cells attenuates TCR- and phytohemagglutinin-mediated activation of the nuclear factor of activated T cells (NF-AT). However, this inhibitory effect does not require SHP-2 association with SIT.

Previous work showed that SHP-1 and SHP-2 as well as SHIP bind to murine FcgammaRIIb phospho-ITIM when co-clustered with BCR (259-261,293). Binding of SHP-2 to the P-ITIM may enhance its catalytic activity, leading to dephosphorylation and release of P-ITIM-bound SHIP and Shc. This SHP-2-mediated dephosphorylation event may negatively control the intracellular localization and/or activity of SHIP and Shc. Another group compared the effect of SHP-1, SHP-2 and SHIP on the inhibitory effect on BCR signaling mediated by Fc gamma RIIB1, using an exogenous expression system, and concluded that SHIP is the major player in this process (340).

SHP-2 seems to be a critical player in suppression of BCR signaling by platelet endothelial cell adhesion molecule-1, PECAM-1, or CD31 (341). Co-ligation of PECAM-1 with BCR on chicken DT40 B cells resulted in a decrease of calcium mobilization, but this was not observed in SHP-2-deficient DT40 variants despite abundant levels of SHP-1 in these cells. However, another group recently reported that both SHP-1 and SHP-2 are both necessary and sufficient to mediate the inhibitory effect of PECAM-1 on BCR signaling (342).

The SHP-2 phosphatase has also been found to interact with the cell surface molecule, SLAM (for signaling lymphocytic activation molecule) (343). SLAM appears to be the human homologue of the mouse cell surface receptor 2B4, an activating NK cell receptor. Notably, SHP-2 and the SH2-containing adapter SAP bind to SLAM competitively. Thus, altered signaling scheme through SLAM might account for the pathogenesis of the X-linked lymphoproliferative syndrome (XLP), which has been mapped to the *SAP* locus.

Adding to the literature are two recent reports yet on another transmembrane protein that binds both SHP-1 and SHP-2 (344,345). MIS or S2V, a novel sialic acid-binding immunoglobulin-like lectin, was able to bind SHP-1 and SHP-2, via the intracellular ITIM motifs. It remains to be clarified whether SHP-1 or SHP-2 (as well as SHIP) plays redundant and/or distinct roles in mediating ITIM functions in various cell types. If SHP-1 and SHP-2 share the same binding site on a specific ITIM motif, it is hard to assign an ITIM inhibitory effect to either SHP-1 or SHP-2. A similar problem exists for signaling of gp130, a common subunit for several cytokine receptors. SHP-1 was shown to bind a tyrosine site on gp130 that mediates an inhibitory effect of mitogenic signals in lymphocytes (346). Thus, it was concluded that SHP-2 is the mediator of this negative effector. However, another group subsequently found that the same tyrosyl residue is also a docking site for suppressor of cytokine signaling-3 (Socs-3) (347), which could complicate the interpretation of previous experimental results.

3.5.2. SHP-2 in neuronal cells

Initial work revealed that SHP-2 was abundantly expressed in the brain (15). However, its function in neuronal cells remains to be elucidated. Stimulation of PC12 cells by nerve growth factor (NGF) leads to tyrosine phosphorylation of BIT (brain immunoglobulin-like molecule with tyrosine-based activation motifs) and its association with SHP-2 (348). Similar observation was also made in primary cultured rat neurons under treatment of brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3). BIT is a membrane glycoprotein that contains two cytoplasmic TAMs (tyrosine-based activation motifs), which apparently mediates the interaction with SHP-2, similar to the ITIM-SHP-2 complex in lymphocytes. Ectopic expression of a phosphatase-inactive mutant (C/S mutant) of Shp-2 in cultured neurons resulted in enhanced tyrosine phosphorylation of several proteins including BIT (349).

Transgenic mice expressing a dominant negative Shp-2 under the control of nestin promoter exhibited an enhanced ischemia-induced damage and neuronal death (350). This observation suggests that SHP-2 is involved in signaling pathway for the control of neuronal survival.

Experimental data have also been presented suggesting a role of Shp-2 in leptin receptor signaling (351-

353). Expression of a catalytically inactive mutant of SHP-2 (SHP-2 C-S) abrogated leptin-stimulated Erk phosphorylation by the long leptin receptor, ObRb, and attenuated egr-1 promoter activation by leptin (354). Mutational analysis suggested that Tyr 985 at ObRb is critical in engaging SHP-2 in mediating Erk activation by leptin receptor.

3.5.3. SHP-2 in mammalian development

Due to the widespread expression pattern of SHP-2, it was not a surprise that a targeted mutation in the murine *SHP-2* gene deleting the SH2-N domain lead to embryonic lethality in homozygotes (355). Multiple defects in the mesodermal patterning and body organization were observed that include abnormalities in the node, notochord and the anterior-posterior axis, poorly developed somites, unclosed neural tubes. However, it is unclear what is the primary defect caused by the SHP-2 mutation. In vitro ES cell differentiation assay and phenotypic analysis of chimeric animals that were derived in part from homozygous SHP-2 mutant ES cells suggest an important role of SHP-2 in the development of erythroid, myeloid and lymphoid cell lineages (338,356,357). SHP-2 also appears to be required at the initial steps of gastrulation, as a signal transducer of FGF receptor (358). In chimeric embryos of embryonic day 10.5, SHP-2 mutant cells accumulated in the primitive streak of the epiblast and failed to contribute to the mesoderm lineage. Chimeric analysis also suggested a specific role for Shp2 in mesenchyme cells of the progress zone (PZ), directly beneath the distal ectoderm of the limb bud (359). The similarity of SHP-2 mutant chimeras to Fgfr1 mutant chimeric limbs supports a notion that FGF-receptor1 and SHP-2 act in concert in a signal transduction pathway within PZ cells. In another study, genetic epistasis as well as chimeric analysis revealed a similar positive role of SHP-2 in signaling downstream of EGF receptor in the development of eye, skin, lung and other organs (360).

3.5.4. SHP-2 in cell migration and apoptosis

SHP-2 was first described as a signaling factor for growth factor and cytokine receptors (15,335). Isolation of targeted SHP-2 mutant embryonic cells led to revelation of a novel function of SHP-2 in the control of cell motility (361). SHP-2 appear s to operate downstream of integrin in the control of cell spreading and migration (361,362). This is consistent with the observations of multiple defects associated with reduced morphogenetic cell movement in SHP-2 mutant embryos and chimeric animals (355,356,358,359). SHP-2 is complexed with focal adhesion kinase (FAK) and possibly modulates the FAK activity during cell migration (361,363). The function of SHP-2 in this process clearly requires its catalytic activity, since expression of a phosphatase-dead mutant of SHP-2 induced a similar phenotype as the SHP-2 defective cells (363,364). SHP-2 may also regulate the cytoskeletal reorganization and cell scattering through modulating RhoA activity (365,366).

Recent work from several laboratories has also suggested participation of SHP-2 in the control of cellular chemotactic responses. Stromal cell-derived factor (SDF)-1alpha stimulation of CXCR4 induced tyrosine phosphorylation in SHP-2 (367). SHP-2 is also associated with the CXCR4 receptor and cytoplasmic signaling molecules SHIP, Cbl, and Fyn. SHP-2 and SHP-1 may also operate in signal relay downstream of CCR5, receptor for macrophage inflammatory protein-1beta (MIP1beta) (368). SHP-2 binds to Tyr763 and Tyr1009 in the PDGF beta-receptor and mediates PDGF-induced activation of the Ras/MAP kinase pathway and chemotaxis (369).

In addition to a positive role in promoting mitogenic response through modulating the Erk pathway, SHP-2 also acts to protect cells against apoptotic effects via several mechanisms. SHP-2 negatively regulates the JAK-STAT pathway induced by interferons (370), and SHP-2 also promotes the activation of the NF-kappaB pathway induced by interleukin–1 and tumor necrosis factor (371). Moreover, Shp-2 appears to mediate v-Src-induced activation of the anti-apoptotic protein kinase Akt (372). Another report indicates that SHP-2 may participate in the protective effect of interleukin-6 against dexamethasone-induced apoptosis in multiple myeloma cells (373).

3.5.5. SHP-2, GABs and SHPS-1 relatives

The key issue for understanding the physiological role of SHP-2 in cell signaling is to identify the substrate(s). Numerous proteins have been found to be associated with SHP-2 in their tyrosine-phosphorylated forms, and these are reasonable candidates as SHP-2 target. Given that SHP-2 is involved in a variety of signaling pathways, it is very likely that this phosphatase has multiple substrates in cells. However, evidence is lacking to clearly assign a protein as a SHP-2 substrate in vivo.

The PH domain-containing scaffold protein, Grb2-associated binder-1 (Gab1), is phosphorylated on multiple tyrosine residues in cells treated with growth factors and cytokines (374,375). Tyrosine-phosphorylated Gab1 is associated with SHP-2 via two phospho-tyrosyl residues at the C-terminal tail and the two tandem SH2 repeats in SHP-2. Several groups demonstrated that this Gab1/SHP-2 interaction is required for Erk kinase activation in response to growth factors (376-378). However, Gab1 does not look like a SHP-2 substrate in cells (378), and similar results were obtained on daughter-of sevenless (Dos), a protein with the same molecular architecture as human Gab1, in *Drosophila* (379,380).

Gab2 is another PH domain-containing scaffold protein that is structurally similar to Gab1 and Dos (381-383). Gab2 seems to be a scaffold protein primarily acting in hematopoietic cells to target SHP-2 to specific substrates in response to IL-3 and other cytokines (381,382,384,385).

A group of transmembrane proteins have been identified that can possibly serve to recruit SHP-2 to the plasma membrane and/or as SHP-2 substrate. The prototype of this family of proteins is SHPS-1 (for SHP substrate 1) or SIRPalpha1 (386,387). Interestingly, SHPS-1 mainly associate with SHP-1 rather than SHP-2 in macrophages (388). A targeted deletion of the intracellular domain of SHPS-1 did not lead to a severe phenotype in

mice (389), but mutant fibroblast cells deficient in SHPS-1 expression exhibited increased formation of actin stress fibers and focal adhesions and spread more quickly on fibronectin, suggesting a role of SHPS-1 in integrin-mediated cytoskeletal reorganization and cell motility. PZR is structurally similar to SHPS-1, albeit with a much shorter extracellular domain (390). The ITIM motifs at the intracellular domain of PZR are not only the docking sites for SHP-2, but also good substrates for SHP-2 in vitro (391). More recently, two groups reported cloning of a novel transmembrane protein, called MIS or S2V that binds to SHP-1 and SHP-2 via the intracellular ITIM motifs (344,345).

Taken together, the primary function of the GAB family proteins and SHPS-1-like transmembrane proteins seems to translocate the SHP-2 tyrosine phosphatase to the membrane. SHP-2 might dephosphorylate these proteins to dissociate from them, but they are apparently not the primary targets.

3.6. PTPases of the PEST-containing group

Three PTPases, PEP (87), PTP-PEST (95-97), and PTP-HSCF (for hematopoietic stem cell factor (98), also known as PTP-K1 (99), PTP20 (100), FLP1 (for fetal liver phosphatase 1, ref. 101), or PTP-BDP1 (for brain derived phosphatase, ref. 102), are currently members of the group of enzymes with sequences enriched in proline, glutamic acid, serine and threonine (PEST). Although PEST sequences have been defined as signature motifs for rapid degradation of cellular proteins by a non-lysosomal, ubiquitination- and ATP-dependent pathway (392), direct measurements indicate that the half-lives of PEST-containing PTPases are quite ordinary; suggesting that the PEST sequences do not target the proteins to accelerated proteolysis (393,394). Instead, these motifs seem to play other roles, such as association with SH3 domains in other proteins by virtue of several Pro-rich stretches within the PEST regions.

Member of this group of PTPases share a common overall structure with an N-terminal catalytic domain and a non-catalytic C-terminal segment of about 500 amino acid residues, which contains the PEST-sequences (figure 1). The extreme C-termini contain a highly conserved 24-amino acid proline-rich region that was proposed to be involved in nuclear targeting at least in some members of the PEST-PTPases (393,101). However, it has been demonstrated that these enzymes are predominantly cytoplasmic (96,99,394,395), and often are enriched near the plasma membrane (396).

The regulation of the catalytic activity of the PEST family PTPases is poorly understood. PTP-PEST has been shown to be phosphorylated in vitro by both cyclic AMP-dependent protein kinase and protein kinase C (397), which are known to have overlapping substrate specificity. The major sites were identified as Ser-39 and Ser-435. Phosphorylation of the former causes a reduction in catalytic activity of PTP-PEST (397). We have also observed that deletion of the non-catalytic C-terminus of PEP resulted in a several fold increase in the catalytic activity (398). This suggests that the holoenzyme may exist in a suppressed state by an intramolecular folding mechanism, similar to that of SHP1 and SHP2. If so, phosphorylation or association with other proteins (e.g. with SH3 domains) may relieve this suppression and cause activation.

The ubiquitously expressed PTP-PEST has been demonstrated to interact, directly or indirectly, with several different proteins including the adapter proteins Shc (399,400), Grb2 (401), Cas (402), Csk (403) and the focal adhesion-associated protein paxillin (404-407). As suggested by this set of proteins, PTP-PEST affects cell adhesion and motility (408,409). It also appears that a primary target for PTP-PEST is the 130-kDa adapter protein Cas (410,411), which interacts with and regulates many key elements of focal adhesions, including c-Src.

PTP-HSCF is preferentially expressed in hematopoietic progenitor cells in the thymus and bone marrow (98,99). In these cells, the enzyme was shown to interact with a SH3-containing member of the actin-associated protein family termed PSTPIP (for proline, serine, threonine phosphatase interacting protein; ref. 412). This cytoskeletal protein is tyrosine phosphorylated in v-*src* transformed cells and is probably dephosphorylated by PTP-HSCF (412). In addition, PSTPTP directs PTP-HSCF to dephosphorylate the c-Abl kinase (413). PSTPIP is homologous to the *Schizosaccharomyces pombe* protein CDC15p, a phosphoprotein involved in formation of the cleavage furrow during cytokinesis (414). The SH3 domain of PSTPIP is also most similar to that of a number of known cytoskeletal regulatory proteins including myosin, spectrin, fodrin, and hematopoietic specific protein (HSP) and cortactin (414). Interestingly, tyrosine phosphorylation of cortactin is enhanced in cells isolated from mice deficient in the Csk kinase (415).

The third member of this group, PEP, is expressed only in hematopoietic cells (87), where it is reported to associate with Csk (395). So far, no other proteins have been reported to interact with PEP, although hematopoietic-specific cytoskeletal protein of the Cas family would be likely candidates. Although the regulation of Csk function is still incompletely understood, it is clear that it inactivates Src family kinases through phosphorylation of their inhibitory C-terminal tyrosine, Tyr-505 in Lck and Tyr-528 in Fyn (5,9,43,416). Csk has been shown to be mainly localized to the cytoplasm with a minor fraction in the plasma membrane (43), but also to focal adhesions in transfected HeLa cells (417,418). Both the SH2 and SH3 domains of Csk were responsible for the targeting to focal adhesions (417). A Csk-binding transmembrane adapter protein termed Cbp (419) or PAG (420) was recently cloned. This protein is localized to plasma membrane lipid rafts and associates with the SH2 domain of Csk by virtue of a phosphorylated tyrosine residue (419,420). Since artificial targeting of Csk to the plasma membrane (by an engineered myristylation site) increased the negative regulatory influence of Csk on Lck and Fyn in T cells and thereby reduced TCR-induced tyrosine phosphorylation and interleukin-2 production

(421), it is can be assumed that Cbp/PAG serves the purpose of bringing Csk (plus PEP) to the plasma membrane to facilitate its suppression of its Src family targets. Following T cell activation, Cbp/PAG is transiently dephosphorylated and Csk dissociates (422), presumably allowing Src family PTKs to maintain a higher level of activity and carry out receptor-triggered phosphorylation events. This model is supported by the finding that cells overexpressing a mutant Csk that lacks the catalytic domain but displaces endogenous Csk from lipid rafts, have elevated basal levels of TCR-zeta-chain phosphorylation and spontaneous IL-2 promoter activation as well as stronger and more sustained responses to TCR triggering than controls (422).

It appears likely that the function of PEP (and perhaps also PTP-PEST) is coupled to the function of Csk. The association and co-localization with Csk already suggest that PEP may dephosphorylate Src family kinases, e.g. at their positive regulatory site (Tyr-394 in Lck), or proteins in physical proximity of these kinases. This site is also the target for the SH2 domain of Csk (423), which may help juxtapose the PEP bound to the SH3 domain of Csk. When PEP is expressed in Jurkat T cells, it does reduce the phosphorylation of Lck at Tyr-394 as well as all downstream signaling events examined (398). The PEP-mediated inhibition of signaling was strengthened by co-expression of Csk (398), even in a cell system where Lck-Y505F was used in place of wild-type Lck that could be directly regulated by Csk. Similarly, the dominant-negative Csk (lacking kinase domain) was even more effective in augmenting TCR signaling when its SH3 domain was mutated to prevent PEP association (422). Taken together, all these observations indicate that Csk and PEP act as a team to suppress Src family kinases. However, more work is needed to define the exact target(s) for PEP in intact cells.

The Csk/PEP module is also the target for an important novel type of regulation that appears to be important in human pathophysiology in conditions that elevate the production of cAMP in lymphocytes, for example in septic shock or HIV infection, and in response to prostaglandin E or cathecholamines. It is well established that cAMP-dependent protein kinase (PKA) type I, which co-localizes with the TCR (424), inhibits T cell activation by phosphorylating one or several proximal targets. A key substrate for PKA is Csk, which upon phosphorylation at Ser-364 is activated several fold in vitro and in vivo (425). This regulation takes place in lipid rafts and results in increased inhibition of Src family kinases and a block in T cell activation. It is not presently known what role PEP plays in this mechanism, but there is a consensus PKA phosphorylation site in PEP. Although a similar site is phosphorylated by PKA in PTP-PEST (397), it is not yet known if PEP indeed is a target for PKA. This is currently under study in our laboratory.

3.7. PTP-MEG2 - a PTPase with a putative lipid-binding domain.

PTP-MEG2 was cloned from a megakaryocytic cell line, MEG-01, and is expressed in many hematopoietic, epithelial, fibroblastic and other transformed cell lines (103). Currently, the 68 kDa PTP-MEG2 is the sole representative of a PTPase containing a 250 amino-acid N-terminus with 28% identity with cellular retinaldehyde-binding protein and 24% identity with SEC14p, a yeast protein with phosphatidylinositol transfer activity (figure 1). The presence of this region prompted P. Majerus and his group, who identified and cloned PTP-MEG2 (103), to suggest that the enzyme interacts with phospholipids, perhaps being regulated by them or being involved in the interaction between phospholipids and tyrosine phosphorylation.

In our laboratory, we have found that PTP-MEG2 resides at the cytoplasmic face of secretory vesicles in hematopoietic and endocrine or exocrine secretory cells (426). Overexpression of PTP-MEG2 in Jurkat T cells caused a striking fusion of these vesicles, ultimately resulting in a solitary vesicular body containing all secretory vesicle markers, such as chromogranin C, carboxypeptidase E, and interleukin-2. This effect required the catalytic activity of PTP-MEG2 and was reversed in minutes by pervanadate (426). Purification of secretory vesicle proteins phosphorylated on tyrosine yielded a putative substrate for PTP-MEG2: the 80-kDa vesicle fusion regulator N-ethylmaleimide sensitive factor (NSF), which was also required for PTP-MEG2-induced secretory vesicle fusion. NSF was found to be phosphorylated at Tyr-83 and a Tyr-to-Phe mutant NSF caused excessive fusion of secretory vesicles, the trans-Golgi network and probably additional intracellular membranes (426).

The N-terminal Sec14p homology domain of PTP-MEG2 binds phosphatidylinositol-3,4,5-trisphosphate in vitro and in intact T cells (426). In cells, disruption of lipid-binding by point-mutations or inhibition of D3-phosphoinositide kinases resulted in inhibition of PTP-MEG2-mediated vesicle fusion (426). These findings suggest that phosphatidylinositol-3,4,5-trisphosphate regulates the function of PTP-MEG2 in the secretory vesicle membrane. Since D3- and D5-specific inositide kinases are known to reside in the Golgi and post-Golgi compartments and regulate the secretory pathway, it appears likely that the physiological function of PTP-MEG2 is to control secretory vesicle biogenesis in response to phosphatidylinositol-3,4,5-trisphosphate made by these kinases (plus a D4-specific kinase). Presumably, this mechanism normally results in secretory vesicles of regular and cell type-specific size, perhaps as determined by PTP-MEG2 expression levels. In support of this model, we have observed that expression of a catalytically inactive PTP-MEG2-C515S mutant caused the formation of smaller than usual secretory vesicles (426).

3.8. The band 4.1 homology (or FERM) domain-containing PTPases

This group of intracellular PTPases currently includes enzymes PTPH1 (104), PTP-MEG1 (105), PTPD1 (106-108), PTPD2 (106), BA14 (109), PTP36 (114; also known as PEZ (115), FAP-1 (110), PTP-BAS, PTP1E, PTPL1, RIP, PTP-BL (111-113). This group of PTPases is characterized by an N-terminal region high homologous to

the cytoskeletal protein band 4.1, followed by a variable central segment, and a C-terminal PTPase domain. In some members of the family, the central segment contains one (PTPH1, PTP-MEG1) or several (PTP-BAS) of the protein-protein interaction domain termed the PDZ (427, 428) (figure 1). Although the physiological function of these enzymes is poorly understood, the analysis of component domains can provide some insight in this area.

The band 4.1 homology domain is an approximately 300-amino acid region first identified in the cytoskeletal proteins ezrin, radixin, and moesin (429). This domain in the band 4.1 protein superfamily (that includes these PTPases) has been termed the FERM domain (in reference to four-point-one protein, ezrin, radixin and moesin). The crystal structure of this domain from moesin (430), radixin (431), and protein 4.1R (432) were recently determined. The structural organization of the domain in these three proteins is markedly similar and comprises three well-defined structural modules that together form a compact clover-shaped structure (433). Each of these modules is structurally homologous to known folds, namely ubiquitin, acyl-coA-binding, and phosphotyrosine binding/pleckstrin folds (434).

The functional role of the FERM (or ERM) domains in ezrin, moesin and radixin is thought to be cross-linking actin filaments with the plasma membrane. A number of studies showed that the FERM domain is involved in cell-cell interaction and membrane trafficking (for review see (434) and references therein). Other than cytoskeleton organization, FERM domains have been found to play roles in signaling pathways (433). Furthermore, the FERM domain enables self-regulation of ezrin, moesin and radixin in which the C-terminus of these proteins binds strongly to the FERM domain leading to their inactivation (434). The functional role of FERM in the FERM-containing PTPases has not been determined to date. However, it is possible that it has the same function of association with the membrane and conformational regulation. For instance, removal of FERM in PTPH1 by limited trypsin cleavage stimulates dephosphorylation activity (435).

Many of the FERM-containing PTPases also contain PDZ domains (427, 428). The central segment of PTPH1 and PTP-MEG1 contains a single PDZ domain, whereas PTP-BAS contains a total of six PDZ domains, one in the extreme N-terminus and five between the FERM domain and the PTPase domain. The PDZ domain from mammalian postsynaptic density protein PSD-95 was crystallized with a bound peptide ligand (436), showing that the structure of the PDZ domain is mainly a beta-barrel. Known PDZ domains show strong preference for large hydrophobic amino acids (e.g. valine, leucine, isoleucine) at the C-terminus of ligands (437). Explanation for this specificity is given by the existence of a hydrophobic pocket within the PDZ domain, which can be filled with large hydrophobic amino acids (438). PDZ domains were also observed to form dimers, possibly by intercalation of β-strands.

It has been suggested that PDZ domains often have a function of molecular scaffolding by holding together multi-protein complexes. Recent findings indicate that PDZ domains may also directly regulate the gating of a transmembrane ion channel (439). The specific function of PDZ domain in FERM-containing PTPases remains still unclear. A study by Cuppen and co-workers (440) showed that the multi-PDZ-containing PTP-BAS binds to a protein known as RIL at an internal segment consisting of a LIM domain (for Lin-11, Isl-1, Mec-3 homology). PDZ domains of PTP-BAS also interact with the Rho effector PRK2 (441) and the RhoGAP PARG (442). It is likely that the PDZ domain(s) in the FERM-family PTPases serve in coordinating interactions with transmembrane proteins, signaling molecules or substrates. In agreement with this notion, it has been found that PTP-BAS/FAP-1 interacts with the cytosolic domain of cell surface receptor Fas/CD95 (443,444).

PTPH1 was also found to associate with an adapter polypeptide 14-3-3β which has high affinity to phosphoseryl-containing motif RSXSpXP (445). Two motifs RSLSpVE (residues 356-361) and RVDSpEP (residues 850-855) in PTPH1 were identified as the binding sites for 14-3-3β (445). PTPH1 was shown to be constitutively phosphorylated at Ser359 and Ser853 in 293 and A431 cell lines, which is required for this PTPH1-14-3-3β binding (445).

The physiological functions of the FERM PTPases have slowly begun to emerge. Several studies reported that PTPH1 is involved in tumor-promoting pathways of different types of cancer. Specifically, a significant increase of expression of PTPH1 mRNA was observed in ovarian (446) and esophageal (447) cancer cell lines compared to normal tissues. However, for liver cancer, Ikuta and co-workers (448) reported no considerable difference in expression levels in PTPH1 versus normal liver tissue. A role in cell growth and morphology was also suggested by two early studies by Gu and co-workers (449,450), who found that expression of PTP-MEG1 in COS-7 cells retarded their rate of proliferation and increased their adherent flattened phenotype. Similarly, expression of PTP36 reduced the rate of cell replication in HeLa cells (451).

The first substrate for a FERM PTPase was recently reported. Using a substrate-trapping mutant of PTH1, N. Tonks' laboratory identified a 97-kDa protein known as valosin-containin protein (VCP) as a physiologically relevant substrate for PTPH1 (452). Another study suggested that PTPH1 may be involved in regulation of membrane fusion and assembly of transitional endoplasmic reticulum (ER) in vitro by dephosphorylating this substrate (453). The tyrosine phosphorylation of VCP triggers its association with ER membrane while PTPH1 action inhibits this association. VCP is also know as a major tyrosine phosphorylated protein in T lymphocytes (454). However, its function in T cells is unknown.

Table 3. Substrate specificity of currently known MKPs

MKP	SUBSTRATE
Pac-1	Erk and p38
MKP-1	Erk, Jnk, p38
MKP-2	Erk, Jnk, p38
MKP-3	Erk
MKP-4	Erk>Jnk=p38
MKP-5	Jnk and p38 alpha and beta
MKP-7	Jnk and p38 alpha and beta
B23	Erk
hVH4	?
M3/6	Jnk and p38
MKP-X	Erk
VHR	Erk, Jnk
VHX	?
TMDP	?
MKP-6	Erk, Jnk

T lymphocytes express four members of the FERM PTPase group: PTPH1, PTP-MEG1, PTP36 and PTP-BAS (396), while PTPD1 and PTPD2 apparently are not present in hematopoietic cells. Of these PTPases, PTP-BAS may participate in Fas-mediated signaling as described above, while the 130-kDa PTP36 may play a role in thymic maturation of T cells as the gene is expressed predominantly in the immature $CD4^+8^+$ cells in the thymus (114). We found that PTPH1, and to a lesser extent also PTP-MEG1, can inhibit TCR-induced signals as measured by Erk2 activation, Jnk activation and the activation of NFAT-AP-1 driven reporter genes (455). The inhibitory effect was dependent of both catalytic activity and on the presence of the N-terminal FERM domain. PTP36 had no effects at all on TCR signaling with these assays.

4. DUAL-SPECIFICITY PROTEIN PHOSPHATASES

The dual-specificity phosphatases (DSPs) are broadly defined as the members of the PTPase superfamily that display activity against other phosphate esters than PTyr. Structurally, DSPs are characterized by an extended and less deep catalytic cleft that allows access of other phosphoamino acids (or phosphoinositol). Many DSPs show preference for adjacent PThr and PTyr in the activation loop of MAP kinases or cyclin-dependent kinases. According to the data that has become available from the human genome project (4) there are 29 DSP genes in the human. Approximately half of these genes have been published, with TMDP (456), hYVH1 (457), MKP-5 (458,459), MKP-6 (460), and MKP-7 (461), being the most recent ones.

In this review, we classify the DSPs in 5 groups according to their structure and other domains present in each enzyme. The first group is the large MKPs (MAP kinase phosphatases), in which we include the known MAP kinase-specific DSPs and their closest homologues. These enzymes are over 35 kDa and contain a regulatory domain amino terminal to the catalytic domain. The second group, the small MKPs, consists of enzymes that are smaller than 200 amino acids with only a phosphatase catalytic domain. They may or may not act on MAP kinases. So far, only 4 mammalian members are known in this group, VHR, VHX, TMDP and MKP-6. Additional members with these characteristics are also present in other organisms, for example VH1 in Vaccinia virus or DMKP (*Drosophila* MKP). Both types pf MKPs share an extended version of the PTPase signature sequence, $VHCXXGXSRSX_5AYLM$. The third group currently consists only of hYVH1 (human yeastVH1), a DSP with an amino terminal catalytic domain and a carboxy-terminal zinc finger domain (457). Cdc25 and KAP form the fourth group related to the MKPs. The fifth group is unique in that the sole member, PTEN, is specific for a non-protein substrate, D3-phosphoinositides, despite being structurally a DSP-like member of the PTPase superfamily and having clear protein phosphatase activity in vitro.

4.1. Large MKPs

This group is defined by the presence of a regulatory domain followed by a catalytic domain. The size of MKPs is variable, ranging from the 34 kDa of PAC-1 to 73 kDa for MKP-7. Many of these enzymes are nuclear (MKP-1, PAC-1, MKP-2 and hVH3) and their expression is induced by growth factors or stress stimuli, whereas the rest of the proteins are either cytoplasmic or nuclear and are not encoded by immediate early genes.

Most of the enzymes belonging to this subfamily display a substrate specificity that cover all types of MAP kinases (See table 3). For example, MKP-1 was originally described as an Erk phosphatase, but was later demonstrated to also dephosphorylate Jnk and p38. It appears that MKP-2, MKP-4 and PAC-1 also are able to act on the 3 main types of MAP kinases. However, it is not clear if the large MKPs are more selective in intact cells, or if substrate selection is regulated by expression, localization, or some type of regulation. On the other hand, Pyst1, Pyst-2 and hVH3 seem to inactivate only Erk, while hVH5, MKP-5, and MKP-7 dephosphorylate only Jnk and p38. From the point of view of the substrates, each MAP kinase apparently can be dephosphorylated by several different MKPs. This stresses the role of MAP kinases as important regulatory integration points in signal transduction.

4.1.1. Large MKP binding to substrates by discrete motifs

An interesting feature of the inactivation of MAP kinases by the large MKPs, is the additional contact the MKPs make with their targets through non-catalytic targeting motifs, which confer both increased substrate association and selectivity towards specific MAP kinases. These motifs are found within the regulatory domains and in the catalytic domains. The currently known motifs that mediate these interactions are the kinase interaction motif (KIM), the LXL docking site for Erk and Jnk (DEJL motif) and the FXFP docking site for Erk (DEF).

The KIM domain is characterized by the presence of 3-4 positively charged amino acids, either Lys or Arg. This motif is common to substrates, activators and regulators of MAP kinases (462), Besides DSPs, KIM regions are also found the PTPases HePTP, PC-PTP and STEP (463), which bind and dephosphorylate MAP kinases. Analysis of the KIM motif by site-directed

mutagenesis in MKP-3 has pointed out that the critical residue is R65, since mutation of this residue alone abolishes binding of MKP-3 to Erk2 (464). Other residues in the KIM of MKP-3 that also contribute to the binding are R64 and L63 (465). This group of amino acids in MKPs interact with a cluster of negatively charged amino acids in the MAP kinases, which constitute a common docking site conserved among MAP kinases (462). It is located near the C-terminus outside the catalytic domain of MAPK and contains two or three acidic amino acids that are adjacent in the steric structure. For example, in Erk2 these residues are D316 and D319 and mutation of D319 results in the *sevenmaker* mutant of *Drosophila* ERK/Rolled. This mutant is less sensitive to inactivation (463).

The DEJL is another motif that mediates the interaction of the MKPs with their substrates and it is present in most of them. This motif was first identified in c-jun as the delta domain (466), later it was found in Elk-1 transcription factor as the D box (467,468) and more recently in MKKs (MAP kinase kinases) (469) and in MKPs (469,470). The consensus sequence for the DEJL motif in MKPs is ILPXLY/FL. The role of this sequence has been tested experimentally in the M3/6 phosphatase, where deletion of the DEJL motif or mutation of L168 and L170 blocked the ability of M3/6 to dephosphorylate Jnk (470). Similarly, in Pyst1, truncation of residues 207-214 abolished the interaction with Erk2 (471).

The third MAP kinase targeting motif, DEF, is found in some MKPs (469), such as MKP-1, MKP-2, Pyst1, Pyst2 and MKP-4, where is found in the C-terminal end of the catalytic domain. DEF sequences are also present in other proteins that interact with MAP kinases, for example the GATA family of transcription factors, the KSR kinases and the Elk subfamily of Ets transcription factors (469). The role of the DEF sequence in MKP-3 (residues 364-367) was demonstrated by mutagenesis, which lead to reduced interaction with Erk2 (464). Additional sequences also participate in MKP - MAP kinase interactions, such as residues 161-177 in MKP-3 (464). A similar sequence is found in two other cytosolic MKPs, MKP-X and MKP-4.

Other domains identified in MKPs are the Cdc25 homology domains (472), CH2A and B. Although the existence of these domains has been known for a long time (472), only recently some data has become available about their function. Site-directed mutagenesis of some residues in the CH2A of the yeast enzyme Ptp3 blocked interaction and dephosphorylation of the yeast MAP kinase Fus3. It is important to note that the CH2A and CH2B regions in Cdc25 flank the active site and function to maintain the structure of the catalytic domain of this enzyme (473), while in mammalian MKPs the CH2 regions are located in the non-catalytic N-terminus.

4.1.2. Catalytic activation of large MKPs by substrate binding

Another interesting feature of the large MKPs is their catalytic activation triggered by binding to MAP kinases (474,475). This mechanism was first described for MKP-3, where it was shown that direct binding to purified ERK2 activated MKP-3 by at least 30-fold (475). According to the prevalent model, MKP-3 exits in two states: a low-activity state in the absence of substrate and a high activity state in its presence. The solution of the crystal structure of the catalytic domain of Pyst1 (human orthologue of MKP-3) (476) has helped to explain the differences between the two states. The Pyst1 catalytic domain has the overall fold of a protein tyrosine phosphatase with a shallow active cleft typical of DSPs to accommodate also the shorter PThr moiety of a substrate. In the absence of substrate, the Pyst1 catalytic domain adopts an open conformation in which the amino acids required for catalysis are not well positioned. For example, Arg-299 that should coordinate two oxygen atoms of the phosphate group is misplaced and Asp-262, that acts as a general acid in the catalysis protonating the tyrosyl leaving group is displaced by almost 5.5 Å from the equivalent position in VHR. These displacements impede catalysis. Binding of Erk2 to Pyst1 induces a conformational change that converts the catalytic domain of Pyst1 into the optimal conformation for catalysis. This model is also supported by the structure of the catalytic domain of PAC-1, which has been solved recently (465).

As discussed above, several motifs are involved in binding of MKPs to their substrates. In the first paper (475) that showed activation of MKP-3 by Erk, it was suggested that deletion of the amino-terminal regulatory domain abolished activation of MKP-3 (465,477). A recent study, however, demonstrated that motifs in the regulatory domain, such as the KIM and residues 161-167 in MKP-3, are also critical for a high affinity binding; whereas the DEJ domain is primarily responsible for Erk-induced MKP-3 activation (464). The same mechanism was found in other phosphatases such as MKP-1 (478), MKP-4 (475) and MKP-2 (479), suggesting that this could be a common mechanism for activating MKP by substrate binding. In intact cells, this mechanism apparently ensures that MKPs remain relatively inactive and harmless to other phosphoproteins until they meet their substrate MAP kinases. This activation upon juxtaposition with substrate seems to be a general type of regulation of PTPases.

4.1.3. Control of large MKP expression in cells

The level of expression of many MKPs is regulated at the transcriptional level. Many of the genes that code for MKPs were described as immediate early genes inducible by growth factors or cellular stresses. This was the case for MKP-1, identified originally as an immediate early gene induced rapidly by mitogens, heat shock or oxidative stress (480,481). In some cell types induction of particular MKPs depend on specific stimuli, for example, in PC12 cells MKP-1, MKP-2 and hVH5 are induced by mitogenic factors, whereas Pyst1 expression is induced by stimuli that promote neuronal differentiation, mainly nerve growth factor (482,483). Regulation at the transcriptional level has also been seen *in vivo*. For instance in rats stimulated with kainic acid MKP-1, hVH3, PAC-1 and MKP-3 are up-regulated in discrete brain regions. Interestingly, different MKPs map to distinct brain areas that show neuronal plasticity, apoptotic cell death or

survival (484,485). MKP-1 also displays transient and highly regionalized induction within specific brain regions during normal embryonic development (484).

The mechanism that control MKP induction are likely to be complex although expression of some gene family members, including MKP-1, MKP-2 and PAC-1, is dependent al least in part on MAP kinase activation (486,487). This provides for negative feedback regulation of the inducing MAP kinase or for regulatory cross talk between parallel MAP kinase pathways.

The levels of MKP proteins are also regulated by postranscriptional mechanisms, such as the degradation in the proteasome. This was illustrated in the case of MKP-1, where phosphorylation of two C-terminal Ser residues, Ser-346 and Ser-364, by p44 MAP kinase led to stabilization of the protein (488). Stabilization of MKP-1 protein did not *per se* affect the ability of this phosphatase to dephosphorylate Erk MAPK.

4.1.5. Physiological roles for large MKPs

Although it seems clear that MKPs target members of the MAP kinase family, it is surprisingly unclear what roles they play *in vivo*. There is only one paper in the literature reporting a MKP knock-out mouse: Dorfman and colleagues deleted the gene for MKP-1, which did not lead to any detectable phenotype or alteration in MAP kinase activation (489). The mice developed normally and were fertile. Cultured cells from these animals did not display any abnormalities in either MAP kinase activation or inactivation (489).

On the other hand, mutations in the *Drosophila* gene *puckered* (puc) that codes for a specific Jnk phosphatase lead to cytoskeletal defects that resulted in a failure in dorsal closure (490). Interestingly, hyperactivation of *Drosophila* Jnk (DJNK) produced by mutations in *puckered* gene or loss of puc expression induced the same phenotype as overexpression of puc: a failure in dorsal closure. This suggests that DJNK must be tightly controlled between certain levels or within certain time-frames. Recently, puc gene has been involved in a new role in *Drosophila*, oogenesis (491).

4.2. Small MKPs

This group of DSPs is characterized by lower molecular weight than the large MKPs, consisting of less than 200 amino acids. The small MKPs only contain a catalytic domain, which has the signature motif for DSPs VHCXXGXSRSX$_5$AYLM, but no recognizable non-catalytic regulatory domains or motifs. So far, only four members of this group have been characterized in mammalian cells, these are VHR (470), VHX (492), TMDP (456) and MKP-6 (460), although several more are expected according to the data available from the human genome project (4). DSPs with the same characteristics have been found in other species, such as the Vaccinia virus phosphatase VH1 or the *Drosophila* MKP (DMKP). The wide distribution of this group of phosphatases suggests that they are involved in essential functions in eukaryotic cells, perhaps unrelated to MAP kinases.

4.2.1. TMDP

TMDP (testis- and skeletal-muscle-specific DSP) was recently cloned from a mouse testis cDNA library by Nakamura and co-workers (456). This protein is expressed solely in testis and skeletal muscle, as its name indicates. Nucleotide sequence analysis of *TMDP* cDNA indicated that the open reading frame of 467 bp encodes a protein of 198 amino acids with a predicted molecular mass of 22.5 kDa. The deduced amino acid sequence contains the signature motif for the catalytic domain of DSPs and shows the highest similarity to human *Vaccinia* HI-related phosphatase (45.5% identity), but a low homology to other DSPs. Recombinant TMDP exhibited intrinsic phosphatase activity towards both phosphoseryl/threonyl and phophotyrosyl residues of myelin basic protein. Expression of TMDP in testis is up-regulated at the meiotic stage during spermatogenesis. TMDP expression in testis is restricted to testicular germ cells, particularly spermatocytes and round spermatids. These data suggests that TMDP may play a role in spermatogenesis.

4.2.2. MKP-6

MKP-6 (map kinase phosphatase-6) was cloned recently by a yeast two hybrid screening looking for novel CD28 cytoplasmic tail-interacting proteins (460). MKP-6 is a protein of 198 amino acids that shares a homology of 22-32 % with the rest of the MKPs. MKP-6 is expressed ubiquitously with high expression in adult trachea, placenta, liver, heart, skeletal muscle, thyroid, fetal thymus and lung. In T cells, expression is up-regulated after TCR plus CD28 cross-linking, both at the mRNA level and at the protein level. MKP-6 inactivates the MAP kinases Erk, Jnk and p38 in vitro, but with a higher specificity for Erk and Jnk. Upon retroviral infection of peripheral blood T cells with an inactive mutant of MKP-6, only Erk and Jnk were hyperphosphorylated. Additional data presented in this study (460), suggested that MKP-6 functions as a negative-feedback regulator of CD28 costimulatory signaling that controls the activation of MAP kinases. Thus, dominant-negative (catalytically inactive) MKP-6 promoted the secretion of substantially larger quantities of IL-2 from infected T cells in response to CD28 costimulation. Furthermore, a chimeric receptor with a mutated CD28 cytoplasmic tail that was unable to bind MKP-6, promoted IL-2 secretion to a much higher extent than a similar chimeric receptor containing a wild-type CD28 tail. Interestingly, another phosphatase that interacts with the CD28 receptor has been described recently (493). In this case, it was a member of the PP2A family of serine/threonine phosphatases. Thus, CD28 may interact with many phosphatases involved in regulating the activation of T cells.

4.2.3. VHR

VHR (for VH1-related) was discovered by S. Aaronson's laboratory by an expression cloning approach (470). The 21-kDa VHR protein was found to dephosphorylate both tyrosine-phosphorylated growth factor receptors and serine phosphorylated caseine in vitro (470). Later, it was shown that VHR dephosphorylates both PTyr and PThr in synthetic phosphopeptides based on the activation loop of the MAP kinases Erk and Jnk (494).

Interestingly, the dephosphorylation of these residues is ordered, first the PTyr residue is rapidly hydrolyzed followed by the slow hydrolysis at the threonine position at a rate which is three orders of magnitude lower. Even more recently, VHR was shown to dephosphorylate Erk *in vitro* but with a slower rate than the Erk specific phosphatase MKP-3 (495).

The human VHR gene was localized to human chromosome 17q21 (496). The VHR mRNA is approximately 4.2 kb and is expressed in most tissues with highest levels in breast, ovary, heart, brain and skeletal muscle (496). The mRNA for VHR is also present in all hematopoietic tissues tested (497). In peripheral blood lymphocytes the protein expression is constitutive and is not regulated by TCR/CD28 costimulation (497).

VHR has been the object of detailed studies to determine its kinetic mechanism, as well as the role of different amino acid residues in this mechanism. Most of these studies have been done using site-directed mutagenesis in conjunction with an extensive kinetic analysis. Like in other PTPases, the conserved active site cysteine (Cys-124) is located at the bottom of the catalytic pocket stabilized by an extensive network of hydrogen bonding (498). The sulfhydryl group of Cys-124 functions as a nucleophile and forms a thiophosphate-enzyme intermediate with the substrate (499). Asp-92 serves as a general acid catalyst for formation of the phosphoenzyme intermediate and in the hydrolysis of thiophosphate intermediate the same Asp-92 acts as a general base (500). Ser-131 has been proposed to donate a proton to stabilize the thiolate-leaving group; mutation of this residue to alanine decreases the rate of phosphoenzyme intermediate breakdown (500). His-123 along with neighboring residues, Tyr-78 and Thr-73, helps to maintain the proper position of Cys-124 in the P-loop (501). The data obtained from the crystal structure of VHR (498) is in accordance with the kinetic data, since the amino acids involved the catalytic mechanism are positioned in the right place to do their job. Thus, VHR follows the same catalytic mechanism as the tyrosine-specific PTPases.

Despite all the data about the kinetic mechanism of VHR, its physiological function is not yet clear. Only recently, a couple of reports have shown that VHR indeed may use MAP kinases as substrates. Todd and coworkers (495) used a substrate-trapping VHR-D92A mutant in an affinity chromatography procedure to show that phosphorylated Erk2 bound to the VHR mutant. When they added VHR to cellular lysates they observed dephosphorylation of Erk. Furthermore they showed that VHR was able to dephosphorylate Erk *in vitro*. Our group has found (497) that VHR regulates T cell activation after TCR/CD28 costimulation. Introduction of exogenous VHR reduced the activation of a nuclear factor of activated T cells and an activator protein-1(NF-AT/AP-1)-driven reporter gene in response to ligation of T cell antigen receptors. In contrast, the activation of a nuclear factor kB-driven reporter was not affected. The inhibitory effects of VHR were also seen at the level of the MAP kinases Erk1, Erk2, Jnk1, Jnk2, and on reporter genes that directly depended on these kinases, namely Elk, c-Jun, and AP-1. In contrast, p38 kinase activation was not affected by VHR, and p38-assisted gene activation was less sensitive. Thus, it appears that VHR may be a bona fide MKP that acts on Erk and Jnk in intact cells. However, it remains to be determined how significant this action is in a cellular context, where other MKPs and HePTP also are present. It also remains unclear if or how VHR is regulated and if it has additional cellular substrates.

4.3. Human YVH1

This DSP differs form the large and small MKPs in that has an N-terminal catalytic domain followed by a zinc finger domain (457), which is not present in any other DSP or PTPase. The mammalian YVH1 was cloned from three different species: human, rat and mouse, having between them a homology greater than 80 %. First, Muda and co-workers cloned the human orthologue, which they called human yeast VH1 (hYVH1) because they were looking for proteins similar to the yeast DSP YVH1 (457). Later, another group cloned the rat YVH1 orthologue from a rat liver cDNA library by interaction with the liver glucokinase, using a yeast two-hybrid screening (502). For this reason, the enzyme was called GKAP (glucokinase-associated phosphatase). More recently, a different laboratory cloned the mouse orthologue (mouse VH1) (503). Additional YVH1-like enzymes, with a catalytic domain followed by a zinc finger domain, have been found in *Drosophila melanogaster*, *Saccharomyces cerevisiae*, *Schizosacharomyces pombe*, *Caenorhabditis elegans* and the malaria parasite *Plasmodium falciparum*. The high degree of conservation of these genes supports the notion that these phosphatases are key regulators of important cellular functions that have been conserved over an extensive evolutionary period. The human YVH1 gene is located on chromosome 1q21-q22, which falls in a region amplified in human liposarcomas

The C-terminal domain of hYVH1 is defined in the study of Muda *et al* as a novel zinc finger domain that is capable of coordinating 2 mol of zinc/mol of protein (457). This domain contains seven invariant cysteines and one histidine reminiscent of sequences observed in the RING, LAP/PHD and LIM motifs. In this work, the authors show that this evolutionary conserved zinc finger domain is essential for *in vivo* function, since the human phosphatase is able to rescue the slow growth defect caused by the disruption of the *S. cerevisiae* YVH1 gene. Surprisingly, the catalytically inactive versions of hYVH1 and VH1 proteins were also able to restore the normal growth phenotype. On the other hand, the C-terminal domain is dispensable for hYVH1 enzymatic activity. The C-terminal domain may target YVH1 to its substrate and this interaction may be sufficient for the physiological function of YVH1.

The study by Munoz-Alonso and colleagues suggests that hYVH1 (GKAP in their terminology) has a function related to glucose metabolism (502). In their study, hYVH1 dephosphorylates glucokinase, an enzyme that phosphorylates glucose at the beginning of glycolysis, accelerating glucokinase activity. Therefore, hYVH1 may

constitute a new regulatory step in glycolysis and subsequently might influence the uptake of glucose in the liver and insulin secretion in the pancreas.

In the mouse, Northern blotting and ribonuclease protection assays revealed that YVH1 has a ubiquitous expression pattern and several splice variants. In addition to the zinc finger domain, a well-conserved aldehyde hydrogenase active site (amino acids 299-310) was discovered by analysis with the prosite program. However, aldehyde hydrogenase activity has not been demonstrated yet. Furthermore, the expression of mVH1 is regulated during the cell cycle, mRNA levels being highest during G1/S transition and declining during the G2/M phase (503).

4. 4. CDC25 and KAP

The eukaryotic cell cycle is regulated by a family of protein kinases, termed CDKs (for cyclin-dependent kinases), which control the progression through the cell cycle at critical check points (504-508). Their phosphorylation state, as well as association with cyclins, control protein kinase activity. The three Cdc25 proteins and KAP are DSPs that control CDKs. Three human Cdc25 genes have been identified, termed Cdc25A, Cdc25B and Cdc25C. They contain the general PTPase signature sequence, but share a limited amino acid-similarity with the PTPases and other DSPs, notably the CH2 region discussed above.

4.4.1 Cell cycle regulation by Cdc25

The Cdc25 proteins remove the inhibitory phosphate from specific threonine and tyrosine residues within the ATP-binding domain of CDKs and, in this way, activate the kinases (504,509). Cc25A appears to activate cyclin E(A)-CDK2 during G1 to S transition, while Cdc25B is involved in the regulation of cyclin A-CDK2 or cyclin A-CDK1 during S to G2 transition (510-512). Cdc25C activates cyclin B-CDK1 at the G2-M boundary (504,513,514). Expression of Cdc25A is controlled by proliferation regulatory signals involving E2F and other transcription factors (515,516).

Cdc25 itself is a phosphoprotein with phosphorylation sites in the N-terminal domain. Chk1 protein kinase, an effector in the DNA-damage checkpoint, phosphorylates Cdc25 at Ser-216 (517,518). Consequently, Cdc25 associates with 14-3-3 proteins and is inactivated, resulting in cell cycle arrest (518-523). Other protein kinases have also been implicated in the regulation of Cdc25: C-Tak1 (524,525), with strong specificity for Ser-216, Cds1 (519), which phosphorylates other sites as well, and ASK1 (apoptosis signal-regulating kinase 1) (504). The C-terminus of Cdc25A binds to ASK1 and inhibits its kinase activity. ASK1 is an upstream component of the kinase cascade that interacts with various stress signals (504,526) - thus increased expression of Cdc25A could contribute to reduced cellular response to oxidative stress under mitogenic or oncogenic conditions, while it promotes cell cycle progression. A particular role has been reserved to Pin 1 which can peptidyl-prolyl-isomerize small phosphopeptides and has been shown to induce a conformational change in Cdc25 with important implications in regulation of cell cycle (527).

The crystal structure of human Cdc25A was independently solved by two groups (528,529) and it does not have the same topology as other phosphatases, being closer to rhodanese structure, which is a sulfur transfer protein (530,527). The overall folding and structure of catalytic subunit of Cdc25B is similar to Cdc25A with several small differences (531).

The activity and proper regulation of Cdc25 is essential for cell proliferation. Cdc25 has been implicated in cell transformation and tumorigenesis, checkpoint control and apoptosis (532). Several reports focused on the role of Cdc25 in the DNA-damage and replication check points (533). Overexpression of Cdc25A shortened the passage through G1 (524,534) while microinjection of anti-Cdc25A antibody inhibited the initiation of S phase (524,511). Thus Cdc25A is involved in a rate-limiting mechanism for G1 progression and initiation of DNA replication (504).

Cdc25A and B are overexpressed in various types of cancers (535-539) resulting in unrestricted cell cycle progression and/or suppressed cell death (504). The levels of Cdc25 mRNA or protein are very different for each depending on the type of tissue and/or cancer (539-542). The important role of Cdc25 in embryogenesis was investigated also in a Cdc25B knockout mouse (534) and in *C. elegans* (535,536). Cdc25 phosphatases cooperate with other oncogenes such as Ha-RAS (524), they are potential targets of at least two oncogenes (Ra-1 and c-Myc) frequently altered in human cancers (524,540,543), they have a tight substrate specificity, a well defined mechanism of catalysis, and their expression is altered in tumors (524). Thus, they deserve special attention as attractive targets for anticancer agents (524).

4.4.2 KAP

The kinase-associated phosphatase (KAP, also termed CDKN3, Cdi1, or Cip2) is also a human DSP that regulates CDKs. Its gene was mapped to 14q22 (544), a region containing abnormalities related to several neoplasms (545-547). It is not yet known whether overexpression of KAP is due to gene amplification or to other mechanisms (548).

The protein kinases and phosphatases that regulate the level of CDKs phosphorylation play essential role in regulation of cell cycle (549). KAP was first identified by two-hybrid screening as a G1- and S-phase phosphatase and further studies implicated it as one of the phosphatases responsible for dephosphorylating the activating PThr residue of human CDK1, CDK2 and CDK3 (550-553). Its rather low catalytic activity (550,551) could be explained by the crystal structure: the presence of a disulfide bridge between the catalytic site Cys-140 and adjacent Cys-79 residue suggesting that the catalytic site Cys is susceptible to oxidation (549).

The dephosphorylation of Cdk2 at Thr-160 is catalyzed by KAP in a cyclin A-dependent manner in vitro (553,554): in the presence of cyclin A, phosphorylated Thr-160 is resistant to dephosphorylation by KAP, and is dependent upon the tertiary structure of CDK2 (549). KAP might act also as a positive regulator for specific Cdks by activating tyrosine-phosphorylated Cdks. KAP/Cd1 interacts with Cdks with different affinities: strongly with Cdc2 and Cdk3 and, to a lesser extent, with Cdk2 (551). Could be that it dephosphorylates also Cdc2 or Cdk3-associated proteins such as cyclins, thus indirectly affecting Cdk activity. It is possible that excess amounts of KAP in transformed cells affect the levels of dephosphorylation of Cdk2 or Cdc2 and their association with their cyclin partners, which results in dysregulation of cell division (548).

KAP expression was found significantly upregulated in breast and prostate cancer both in vitro and in vivo (548). In normal cells KAP was primarily localized in the perinuclear region, but in tumor cell a significant portion was in cytoplasm. Blocking KAP overexpression resulted in a decreased population of S-phase cells during cell cycle progression and reduced Cdk2 kinase activity. In this way KAP is playing a role in normal growth control and deregulated KAP expression may contribute to the malignant phenotypes (548). It is possible that KAP overexpression might also be associated with the overexpression of a specific oncogene in these types of cancer (548).

4. 5. PTEN

The *PTEN* (for phosphatase and tensin homologue deleted on chromosome 10 (75), also called *MMAC1* for mutated in multiple advanced cancers 1 (76), or *TEP1* for transforming growth factor ß-regulated and epithelial-cell-enriched phosphatase 1 (555)) gene was originally identified in 1997 as a human suppressor gene located on chromosome 10q23.3, a genomic region that suffers loss of heterozygosity in many human cancers (556-558) and its importance has been validated by identification of mutations and/or loss of expression in almost half of all endometrial cancers, a third of glioblastomas and at lesser frequencies in a wide range of other human neoplasms such as prostate, brain, breast and kidney cancers or melanoma (75,76,555,559-563). Abnormalities at 10q are also found in many lymphoproliferative diseases and mutations of *PTEN* have been demonstrated in leukemic cell lines (564,565). Its altered expression usually correlates with the late stages of the diseases. Germline mutations in *PTEN* are also associated with Cowden disease, Bannayan-Zonana syndrome and Lhermitte-Duclos disease (558,566-569) which are autosomal dominant disorders with specific developmental defects, multiple benign tumors and increased susceptibility to breast and thyroid malignancies. Mice lacking PTEN show overgrowth of the cephalic and caudal regions and are embryonic lethal (570-572). Heterozygous *PTEN* $^{+/-}$ mice are viable but have autoimmune disorder, defective Fas-mediated apoptosis and an abnormally high rate of cancer (573).

The *PTEN* gene encodes a 403 amino acids protein (~55 kDa) with the hallmarks of a DSP in its N-terminal catalytic domain, which also has some homology with the cytoskeletal proteins auxilin and tensin (75,574,575). The C-terminus consists of a lipid-binding C2 domain, a regulatory PEST region and a C-terminal tail that can bind PDZ domains. The catalytic domain contains the PTPase signature sequence and has an overall fold similar to PTPases and DSPs. Indeed, in vitro PTEN can dephosphorylate both PTyr and PThr/PSer containing substrates, but the specific activity is quite low unless extremely acidic substrates are used (576). Instead, PTEN readily dephosphorylates phosphatidylinositol (3,4,5)-trisphosphate and phosphatidylinositol (3,4)-bisphosphate specifically at the D3 position of the inositol ring (78). A good number of papers now agree that these lipids are the physiologic substrate of PTEN (78,558,576-578).

The crystal structure of the catalytic domain of PTEN strongly supports the notion that D3-phosphoinositides are the true substrates for PTEN. The catalytic cleft is significantly wider for PTEN, although similar in depth, so it can accommodate the large, multiply phosphorylated inositol head group of the substrate and the three positively charged amino acids (Lys-125, Lys-128 and His-93) which surround the catalytic site and determine the preference for highly acidic substrates (574,575).

C2 domains are known to have lipid-binding and membrane targeting functions (574,579). Two of the three C2 domains loops contain basic residues and this predicts a direct contact of PTEN with the membrane (574), unlike the majority of C2 which get through to the membrane by their acidic residues bound to Ca^{2+}. The C2 domain of PTEN has also been shown to promote access of the catalytic domain to the phospholipid substrate. Mutations in the C2 domain have been shown to impair the tumor suppressor function of PTEN. There are two categories of C2 mutations: mutations disrupting the hydrophobic beta-sheet core and mutations at the level of the loops connecting the beta-strands. The former type results in decreased phosphatase activity (579) and the later variably impairs the stability of the protein (580). The most frequent genetic events affecting the C2 domain are C2 truncation mutations with total loss of tumor suppression (579). This was seen as tumor formation, anchorage independent growth of the cells and a significantly impaired ability of the mutant proteins to bind to lipid vesicles in vitro or to inhibit the growth (depending on the cell type) in PTEN-deficient lines (even if their lipid phosphatase activity was intact). Thee mutants were also unstable and rapidly degraded (579). It is possible that some of these mutants also affect the binding of PDZ domain-containing proteins and thereby affect the regulatory influences of such proteins, such as PTEN degradation and its ability to inactivate PKB (581, 582).

The C-terminal tail of PTEN contains the motif TKV, which fits the ligand binding consensus for many PDZ domains. Indeed, using the yeast two-hybrid system, PTEN was found to associate with the multi-PDZ proteins MAGI-2 and MAGI-3 (for membrane associated guanylate kinase family with multiple PDZ domains, localized to epithelial cell tight junctions) (558,581-584). This

association enhances the efficiency of PTEN function, presumably by affecting its membrane localization. PTEN mutants that fail to bind MAGI proteins show defects in Akt regulation (558,581,582). Furthermore, the addition of a PDZ domain to a truncated catalytically inactive PTEN mutant conferred a greater degree of stability to this protein, which otherwise was rapidly degraded (581). More specifically, dephosphorylation of PTEN at Thr-382 and -383 increased the affinity for MAGI-2 (15-30 fold) most likely through the second PDZ domain and shortened the half-life of the mutants (585). Therefore, this type of protein interactions, even if it is not strictly necessary for tumor suppression, appears to improve the efficiency of PTEN and its function in intact cells (583). For example, the intact C-terminus of PTEN is essential for its regulation of PDGF induced membrane ruffling in Swiss 3T3 cells, which depends on D3-phosphoinositides and the Rac GTPase, but not PKB (586). In this way, it seems quite likely that the effects of PTEN on PKB and Rac–induced responses (considered as distinct pathways downstream of PI3K) might have different requirements for PDZ domain-dependent targeting of PTEN (586).

Upstream of the PDZ-binding tail of PTEN is a 50-residue region that is rich in Pro, Glu, Ser and Thr (PEST). Although this region is dispensable for the tumor suppressor function of PTEN (579), it has a strong influence on the degradation and intracellular half-life (see Chapter 3.6) of PTEN. It appears that in vivo most of PTEN is phosphorylated with this region and remains relatively stable, but functionally inert (583,585,587). Upon dephosphorylation, PTEN becomes more active, but also rapidly degraded (581). The phosphorylation sites have been mapped by several groups (583,585,587), and appear to include Ser-380, Thr-382, Thr-383, and Ser-385. Mutation of some of these to an alanine reduced the steady-state PTEN protein levels by shortening their half-lives. These mutants (as well as the “tailless” protein) were also more active in inducing G1 arrest and FKHR transcriptional activation (583). Casein kinase II has been suggested to be the protein kinase responsible for the phosphorylation of PTEN (587).

Since PTEN is primarily, perhaps exclusively, a D3-phosphoinositide phosphatase, the physiological functions of PTEN are intimately associated with those of phosphatidylinositol 3-kinase. The reported roles include tumor suppression, blocking cell cycle progression and proliferation, reducing cell migration and inducing apoptosis (557,575,577). The D3-phosphoinositide substrates for PTEN (569,588) mediate the translocation of Akt (also called protein kinase B, PKB) to the membrane inducing a conformational change which allows PDK1/PRK-2 to phosphorylate and, consequently, activate Akt (577). By dephosphorylating these lipids and preventing the activation of PDK1 and Akt, PTEN plays an essential role in counteracting antiapoptotic signaling. This is best seen as decreased levels of phospho-Akt in cells expressing active PTEN (557,589-591). Conversely, mutations that inactivate PTEN (e.g. in tumors) result in accumulation of D3-phosphoinositides, which promotes unrestrained signaling through Akt and increased cell survival and proliferation.

PTEN also affects cell cycle progression. When low levels of PTEN are reintroduced into PTEN-/- embryonic stem cells and in a variety of PTEN deficient cell lines, the cells generally arrest in the G1 phase of the cell cycle (588,592-594). This effect requires PTEN lipid phosphatase activity. PTEN-mediated downregulation of Akt stimulates transcription of cyclin-dependent kinase inhibitors (p27Kip1, p21waf1 and p57Kip2) by Forkhead family transcription factors (595), thereby inhibiting cyclin E/CDK2 activity and specifically reducing cyclin D3 levels (577,596). This leads to cell cycle arrest in late G1 phase (563,577,595-602). PTEN has also been suggested to cause G1 arrest by inhibiting the hyperphosphorylation and function of the retinoblastoma protein (603).

In other cells, and presumably at higher levels of expression, PTEN induces rapid induction of programmed cell death by apoptosis (604,605) or G1 arrest followed by apoptosis (606). PTEN also renders glioma cells more susceptible to anoikis (apoptosis initiated by disruption of cells’ interaction with the extracellular matrix) (586). In a myeloid leukemia cell line the induction of apoptosis or G1 cell cycle arrest and expression of p27kip1 were achieved by administrating vitamin D3 compounds which led to upregulation of PTEN expression (607).

In some cell lines, PTEN expressed at normal levels did not affect the growth in vitro nor the proliferative rate. Instead there was a dramatic difference in the tumor vascularization and its volume: tumors derived from cells expressing PTEN had fewer blood vessels than those derived from the parental line or cell lines expressing PTEN mutants. PTEN reconstitution diminished phosphorylation of Akt, induced trombospondin 1 expression (an inhibitor of angiogenesis) and suppressed angiogenic activity (608). Wen and co-workers (608) emphasized that most cell lines and tissues express PTEN without undergoing G1 arrest, so this feature, detected by many groups in their studies, may only be the result of overexpression of PTEN. All these effects - G1 arrest, apoptosis, and inhibition of angiogenesis - required PTEN to have an intact lipid phosphatase activity (592,608,609). The growth suppressive effect of PTEN is achieved through the inhibition of two separate signaling pathways, the PI3K pathway and the MAPK pathway depending on the cell signaling context (610,611).

The relationship between PTEN and insulin signaling has been studied in many cell types and organisms. In 3T3-L1 adipocytes, PTEN significantly inhibited insulin-induced PI3K dependent processes such as GLUT4 translocation, glucose uptake and membrane ruffling, phosphorylation of Akt and p70S6 kinase. Not all of the actions of insulin were inhibited: stimulation of MAP kinase was unaffected by PTEN overexpression (612). In 293 cells overexpressed PTEN reduced the levels of insulin-induced PIP3, without affecting insulin induced PI3kinase activation. Oligonucleotide chip technology revealed that IRS-2 (insulin receptor substrate-2) gene is upregulated by PTEN, being the most induced gene at all time points (613). PTEN expression also augmented insulin-promoted tyrosine phosphorylation of IRS-1 and diminished the ability of TNF to suppress this effect (614).

In a breast cancer model, after insulin stimulation, PTEN seemed to prevent IRS1/Sos/Grb2 complex formation, most likely due to decreases in IRS-1 phosphorylation (610). This blocked MAP kinase phosphorylation, downregulated cyclin D1, inhibited cell cycle progression and suppressed cell growth. The authors suggested a differential two-phase phosphorylation of IRS1 in response to insulin stimulation: tyrosine phosphorylation occurs initially and then a delayed serine/threonine phosphorylation, PTEN affecting only the later phase (610). The *C. elegans* DAF-18 gene is a close homologue of PTEN and acts as a negative regulator of the insulin signaling pathway in this organism (615-618). *Drosophila* PTEN, on the other hand, affects cell size, cell cycle progression and apoptosis and also acts as an inhibitor of the insulin signaling pathway (619-623).

PTEN expression inhibits also, strongly and relatively specifically, both IL-1 and TNF induced DNA binding and the transcriptional activities of NF-kappaB (624,625), induces TIMP3 (inhibitor of matrix metalloproteinases 3), suppressing matrix degradation in vivo (608), and blocks membrane ruffling (e.g. in Swiss 3T3 cells upon exposure to PDGF). Impaired PTEN activity increased HIF-1a (hypoxia-inducible factor 1a) expression (626) and stimulated VEGF production (627). The PPARgamma, which is involved in inflammatory responses, has been implicated in the regulation of PI3K signaling by modulating PTEN expression in inflammatory and tumor derived cells (586) whereas in leukemic cell lines a negative regulatory role in TCR stimulation was proposed for PTEN (578,628).

Although PTEN clearly acts as a D3-specific phospholipid phosphatase, it has also been claimed to dephosphorylate a protein substrate: focal adhesion kinase (FAK). Since PTEN plays a role in cell attachment and in anchorage-dependent cell survival, it is not surprising that PTEN affects FAK (629,630). However, the proposed direct dephosphorylation of FAK by PTEN (631) probably is not physiologically relevant: there was no difference in the phosphorylation status of FAK in PTEN -/- versus PTEN +/+ ES cells (588). Furthermore, while the suppression of cell growth (632), focal adhesion formation (631) and cell migration and invasion (629) observed after expressing PTEN cDNA in PTEN deficient glioblastoma cells or fibroblasts (631,633) needed a functional phosphatase catalytic domain, a G129E mutant PTEN, which lacks lipid phosphatase activity, but retains protein phosphatase activity, does not suppress tumor cell growth in soft-agar, nor does it induce cell-cycle arrest. (557,592,609,631,632). However, this mutant does retain cell spreading inhibitory activity and also results in dephosphorylation of FAK. Thus, some functions of PTEN may be independent of PI3K and D3-phosphoinositides (557).

Other studies implicated PTEN in the dephosphorylation of Shc, which inhibited the recruitment of Grb2 and downregulated the MAP kinase pathway (634,635). On the other hand, PTEN expression can inhibit the movement of Gab1 to the plasma membrane by dephosphorylating D3-phosphoinositides since the pleckstrin homology domain of Gab1 binds this phospholipid to translocate to the membrane (636). This inhibition may block signaling through the MAP kinase pathway without needing direct dephosphorylation of Shc. Differences between PTEN mutants and PI3K were also observed in *Drosophila*, where Goberdhan and co-workers (619) noted that some of the phenotypes observed in dPTEN mutants have not been reported in Dp110 or insulin-signaling mutants, suggesting that these effects are not caused simply by an increase in D3-phosphoinositide levels and that the role of PTEN could be broader than just dephosphorylation of these lipids. The observation that some PTEN protein is located in the cell nucleus may also relate to additional functions (637).

PTEN is essential for embryonic development, possibly by regulating cell proliferation (570-572,619,620,638). During human development high levels of PTEN expression are observed in the skin, thyroid, central nervous system, autonomic nervous system and upper gastrointestinal tract (639). PTEN may contribute to cell differentiation by antagonizing PI3K (640, 641). Its expression being significantly induced by specific differentiation inductors (607).

The BLAST searches of human genomic sequences resulted in at least one PTEN pseudogene (642) and two distinct PTEN-related genes both with possible specificity for lipid substrates (577,643). PTEN2 is a highly conserved homologue of PTEN which is Golgi-localized testis-specific phosphatydylinositol phosphatase and may contribute to the terminal stages of spermatocyte differentiation (644).

In summary, PTEN has gained enormous popularity as a research target in recent years. In less than five years, PTEN has clearly established its position as the most mutated gene in human cancers after p53, with huge implications in cell life and death of cells. This gene shows great promise as in the diagnosis of the earliest stage of nonfamilial malignancies and in the development of new therapies to treat human disease.

5. LOW MOLECULAR WEIGHT PTPases

5.1. LMPTP

The low molecular weight PTPase, LMPTP, was originally purified from liver by Heinrikson as an acid phosphatase (645) and was subsequently purified to homogeneity and its amino acid sequence determined (646,647). Even before its cDNA was isolated (72,648), it was clear that several isoforms or related enzymes exist. It was subsequently found that the primary transcript undergoes an alternative splicing event in which either exon 3 or exon 4 is excised (649). This results in two different isoforms: LMPTP-A, which contains exon 3 and moves a bit slower on SDS gels, and the faster moving LMPTP-B, which contains exon 4-encoded amino acids. We have also detected a third splice form, which lacks both exon 3 and exon 4 (650). This isoform, which we term LMPTP-C, is only 15-16 kDa and lacks catalytic activity *in*

vitro. The corresponding mRNA can be detected in all tissues and cell lines examined, and appears to be as abundant as the other isoforms. The LMPTP-C protein can also be visualized by Western blotting (650). The LMPTP gene is highly conserved through evolution from yeast to man. The homologues from budding yeast (*Saccharomyces cerevisisae*), Ltp1 (651), and fission yeast (*Schizosaccharomyces pombe*), Stp1 (46526), are 39% and 42% identical to the mammalian enzyme, respectively. Recently (653), a homologous gene was found in the prokaryote *Acinetobacter johnsonii*. The protein was found to have PTPase activity in vitro and to dephosphorylate a novel bacterial PTK that autophosphorylated on tyrosine (653). This suggests that LMPTPs are likely to be involved in the regulation of an ancient and perhaps fundamental cellular function. On the other hand, disruption of the two yeast genes did not cause any observable loss of viability or phenotypic chances (651,652).

Already in 1989, G. Ramponi's group showed that LMPTP readily dephosphorylates PTyr in a protein substrate as well as free PTyr, while not hydrolyzing PThr or PSer (654). LMPTP also dephosphorylates other aromatic phosphates *in vitro* (655), and is not yet entirely clear if LMPTP acts only as a PTPase in intact cells. The amino acid sequence of LMPTP showed that the enzyme contains a minimal PTPase signature sequence, which is the only recognizable homology to other PTPases, and which is found in a unique location in the sequence: in the extreme N-terminus (figure 2). LMPTP is also remarkably small, only 18 kDa, which is less than the mere PTPase domain of classical PTPases. The crystal structure of LMPTP-A (656) and LMPTP-B (657) reveal that the cysteine residue (C12) of the signature sequence is located in a catalytic pocket much like in other PTPases, while the rest of the enzyme has a unique folding. Cys-12 was already known from biochemical studies to be crucial for catalysis (655,658). The amino acids encoded by exons 3 or 4 (referred to as the 'variable loop') form one side of the catalytic pocket, indicating that the two isoforms are likely to select substrates with different amino-acid residues next to the target PTyr. The two isoforms also differ in catalytic properties and stimulation by nucleotides (658).

The biological function of LMPTP is still unclear. A number of reports by G. Ramponi's group suggest that highly overexpressed LMPTP negatively regulates cell growth and transformation by oncogenes (659,660) and counteracts the platelet-derived growth factor (661,662) and insulin (663) receptors by dephosphorylating specific tyrosine residues on these receptors. Recently also one of the VEGF receptor (Flk-1) has been proposed as a possible physiological substrate of LMPTP-A (664). Also in our hands (650), overexpression of LMPTP-A or -B in NIH3T3 cells causes some reduction in growth factor-induced gene activation. Interestingly, the inactive LMPTP-C isoform had the opposite effect, suggesting that it might compete with endogenous LMPTP in a dominant negative fashion.

LMPTP has been also found to interact with oligomeric eph receptors and such interaction could play a role during the development of central nervous system (665). Indeed the Drosophila Primo locus which shows a very particular pattern of expression in the developing retinal tissue was found to codify for the two isoforms of Drosophila LMPTP (666).

We have found that LMPTP-B is phosphorylated on tyrosines 131 and 132 in T cells and that such phosphorylation *in vitro* activates the enzyme (18). In co-transfected COS cells, the T cell Src-family kinase Lck was particularly efficient in phosphorylating LMPTP. A recent paper by Rigacci and co-workers reported that LMPTP-A is tyrosine phosphorylated in v-Src transformed NIH3T3 cells, and that Src was able to phosphorylate LMPTP *in vitro* (19). Interestingly, the C isoform was not phosphorylated by Lck in co-transfected COS cells or even *in vitro*, despite still having the two tyrosines. This indicates that the three-dimensional folding of LMPTP is important for the recognition of Tyr-131 and Tyr-132 by Lck. These two residues are next to the catalytic pocket and it is conceivable that their phosphorylation affects the interaction of LMPTP with substrates. The amino acid residues encoded by exons 3 or 4 form the other rim of the catalytic cleft, and are therefore likely to affect substrate binding.

LMPTP is one of very few tyrosine phosphatases which are known to be genetically polymorphic: three alleles have been identified at the LMPTP locus (still called ACP1 locus) (667,668): the genetic polymorphism affects the mRNA splicing (669) resulting in different total activity and A/B ratio between the alleles. LMPTP genetic polymorphism has been used for many years as a forensic marker and the LMPTP locus -located on chromosome 2p25- is relatively well known compared to the those codifying for other PTPases (670). LMPTP polymorphism has been and is still being extensively studied for association with human diseases and seems to have a role in the predisposition to several common human diseases (671).

In conclusion, LMPTP is a small phosphatase with strong preference for PTyr over PSer or PThr, but with somewhat unclear substrate specificity in intact cells. The specificity and activity of the enzyme are regulated by alternative splicing and tyrosine phosphorylation, but its physiological substrates and biological function remain unclear.

5.2. PRL-1, 2 and 3 - isoprenylated nuclear enzymes

PRL-1, 2, and 3 are a subgroup of closely related small PTPases that contain a C-terminal prenylation motif, CAAX (672), similar to that of the Ras family of proteins. PRL-1 (phosphatase of regenerating liver-1) was the first gene of this family identified as an immediate early gene in proliferating hepatocytes (73). This phosphatase has been proposed to be associated with the control of cellular growth and differentiation (73,673). Although these phosphatases were first found in the nucleus (73,672, 673), recent work shows that they are present on the plasma membrane (674, 675) and in intracellular punctuate structures (674). Besides, intracellular localization of these

proteins depends on their prenylation. Thus, prenylated proteins tend to localize to the plasma membrane, while in the absence of prenylation the PRL phosphatases go to the nucleus (674).

Although the physiological function of the PRL phosphatases is still not determined, recent work has made some advancements. Using the yeast-two hybrid technique to look for PRL-1 interacting proteins, Peters and co-workers (675) found a novel basic leucine zipper protein, ATF-7, which is related to members of the ATF/CREB family of transcription factors. PRL-1 was shown to be able to dephosphorylate ATF-7 *in vitro*. Another report described the interaction of PRL-2 with the beta subunit of Rab geranylgeranyltransferase II (676). This interaction was specific of PRL-2, since PRL-1 and PRL-3 did not bind to geranylgeranyltransferase II. Thus, PRL-2 may regulate geranylgeranyltransferase II. Transgenic mice selectively overexpressing PRL-3 in the heart show overt cardiac hypertrophy and reduced cardiac function associated with impaired calcium handling (677). Furthermore, it was shown in another study that PRL-3 overexpression in HEK293 cells blocked the release of intracellular calcium after angiotensin II stimulation (678). Taken together, these studies indicate that the three PRL enzymes may each have different functions that relate to transcription, intracellular traffic and signal transduction.

6. CONCLUDING REMARKS

Our understanding of the role of tyrosine phosphorylation in the regulation of transmembrane signaling, cell proliferation, differentiation, cytoskeletal architecture, apoptosis, and intracellular vesicle traffic has improved greatly during the past few years. Still, many important questions remain open, particularly with regard to the PTPases that both counteract the PTKs and regulate them. Given the central role of tyrosine phosphorylation in many fundamental cellular processes, a deeper understanding of these molecular events would greatly aid the design of rational pharmacological approaches to treat human disease. This is particularly evident for the immune system, where pharmacological agents could augment the defense against pathogens, modulate autoimmune disorders (679,680), including multiple sclerosis (681) and interfere with the growth of lymphoid malignancies. The known crystal structure and small size of many PTPases make them ideal targets for rational drug design. Before these enzymes can become sufficiently attractive as targets for the pharmaceutical industry, however, we must gain considerably more insight into their physiological functions and importance. Their substrate specificity and selectivity, their redundancy and their regulation also need to be explored. The presence of quantitative genetic variability and its possible effects on physiological and pathological parameters may also give important information to guide pharmacological approach.

Lymphocytes express many PTPases, only some of which have currently understood functions. These include CD45, SHP1 and HePTP. There is recent evidence (682), however, that T cell membranes contain a PTPase activity that efficiently keeps the TCR signal transduction machinery, including the Src and Syk family PTKs, in a resting phosphorylation state. Inhibition of this activity, which was not attributable to CD45, SHP1 or HePTP, induced the same ordered sequence of phosphorylation events as normally seen after TCR ligation (682). It remains to be seen if this membrane-bound PTPase is identical to one or several PTPases discussed in this review or yet another novel enzyme.

A very exciting new area of investigation is the involvement or loss of PTPases in human malignant diseases. The surface expression of CD45 has long been used as a marker for differentiation of leukemic cells (683,684). There is no evidence, however, that CD45 would be directly associated with the transforming event or progression of leukemias or lymphomas. Instead, loss of CD45 causes severe immunodeficiency in patients (685,686). The first examples of a potential role for a PTPase in neoplasia was the finding that HePTP might be overexpressed in myelodysplasia and deleted in leukemias (213). With the recent elucidation of the MAP kinase inactivating function of HePTP, these suggestions make sense. The now well established fact that PTEN is deleted or mutated in multiple human cancers (see Chapter 4.5), and that Cdc25 is overexpressed in many cancers (see Chapter 4.4.1), add to the notion that PTPases are likely to involved in clinically relevant carcinogenesis. Finally, loss of MKP-1 expression or activity was found to be associated with malignant transformation and clinical progression in a subset of epithelial tumors (687). As overexpression of many PTKs can cause malignant transformation, it is natural to assume that loss of the PTPases that counteract them would also cause neoplasia. Furthermore, genetic damage is perhaps more likely to cause deletion or loss of function rather than overexpression and gain of function. We predict that several PTPase genes will be found in the near future to be altered in human malignancies. This underscores the importance of studying these enzymes and the value of increased understanding of their regulation and substrates for a rational drug development to treat human diseases.

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