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Protein tyrosine phosphatases in health and disease

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Protein tyrosine phosphatases (PTPs) represent a super-family of enzymes that play essential roles in normal development and physiology. In this review, we will discuss the PTPs that have a causative role in hereditary diseases in humans. In addition, recent progress in the development and analysis of animal models expressing mutant PTPs will be presented. The impact of PTP signaling on health and disease will be exemplified for the fields of bone development, synaptogenesis and central nervous system diseases. Collectively, research on PTPs since the late 1980's yielded the cogent view that development of PTP-directed therapeutic tools is essential to further combat human disease.

Abbreviations
AChR, acetylcholine receptor; Akt/PKB, Akt/protein kinase B; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CNS, central nervous system; CNTN-1, contactin-1; CSPG, chondroitin sulfate proteoglycan; cyt, cytosolic; DLAR, ortholog of the mammalian LAR family, type IIa RPTPs; DUSP, dual-specificity PTP; EAE, experimental autoimmune encephalomyelitis; EKO, PTPε knockout; FERM, 4.1 protein/ezrin/radixin/moesin; FNIII, fibronectin type III; GRIP, glutamate receptor interacting protein; HD-PTP, His-domain containing PTP; HSPG, heparan sulfate proteoglycan; IL1RAPL1, interleukin-1 receptor accessory protein-like 1; JNK, c-Jun N-terminal kinase; LAR, leukocyte common antigen related; LTD, long-term depression; LTP, long-term potentiation; LYP, lymphoid tyrosine phosphatase; MAP, mitogen-activated protein; MAPK, mitogen-activated protein kinase; M-CSF/CSF-1, colony stimulating factor 1 (macrophage); (m)GluR, (metabotropic) Glutamate receptor; MKO, Mkp-1 knockout; MS, multiple sclerosis; MuSK, Muscle specific kinase; NGL-3, netrin-G ligand-3; NMDAR, *N*-methyl-D-aspartate receptor; NMJ, neuromuscular junction; OLG, oligodendrocyte; OPC, oligodendrocyte precursor cell; PDZ, PSD95/disc large tumor suppressor/zona occludens protein; PEZ, PTP/ezrin-like; PSD95, post-synaptic density protein 95; PSTPIP, proline serine threonine-rich phosphatase interacting protein; PTEN, phosphatase and tensin homolog; PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; PTP-BL, PTP-BAS-like; PTP-PEST, PTP with proline/glutamate/serine/threonine-rich domains; Pyk2, product of PTK2b gene; RANKL, receptor activator of nuclear factor kappaB ligand; RhoGAP, Rho GTPase activating protein; RPTP, receptor-type PTP; SAP-1, stomach cancer-associated PTP-1; SFK, Src-family tyrosine kinase; SH2, Src homology type 2; SHP, SH2 domain containing PTP; SNP, single nucleotide polymorphism; STEP, striatal-enriched phosphatase; TCPTP, T-cell PTP; TCR, T-cell receptor; TGF-β3 transforming growth factor β3; WASP, Wiskott-Aldrich Syndrome protein.

Introduction

The reversible phosphorylation of proteins is one of the most powerful ways by which living organisms orchestrate the development and function of their constituting cells. Cellular protein phosphotyrosine levels are tightly regulated by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Members of the super-family of PTPs catalyze the dephosphorylation of phosphotyrosine, many PTP family members [known as dual-specificity phosphatases (DUSPs)] also remove phosphate groups from phosphothreonine and phosphoserine residues in proteins and some PTP family members dephosphorylate phospholipids, phosphorylated carbohydrates or oligonucleotides [1, 2]. PTP substrate specificity and activity is tightly regulated by various mechanisms ([3] and elsewhere in this issue). Research over the past decades has firmly established important and diverse roles for PTPs in cellular signaling pathways, embryonic development and disease. Most convincingly, in several cases mutations in PTP genes have been shown to be causative for, or to contribute to, the penetrance or severity of the disease state. For recent and extensive reviews on alterations of PTP genes in somatic cells, including mutations or dysregulated expression, as observed in human diseases such as cancer or metabolic disorders, we refer to other publications [4–12]. Here, we will first briefly list the PTPs that are associated with hereditary human pathologies. Subsequently, we will highlight the powers of distinct animal models in contributing to our knowledge on how PTPs exert their actions in (patho)biological conditions. Finally, we will review the role of PTPs in bone and synapse development and in central nervous system (CNS) disease states.

Over the past 25 years, the PTP field has evolved and it is evident that PTPs have essential roles in many biological processes. The authors of this review all collaborated in a European Union-funded Marie Curie Research Training Network, PTPNET, which ran from 2007 to 2011. The topics that we highlight in this review reflect the research interests and expertise of the co-authors.

PTPs in hereditary human disease

Hereditary disease phenotypes that are caused by mutations in classical PTP genes have been described to date for *PTPN1*, *PTPN2*, *PTPN11*, *PTPN14*, *PTPN22*, *PTPRC* and *PTPRQ* (Table 1). Interestingly, both loss-of-function and gain-of-function mutations in these genes may underlie the reported disease. *PTPN11* is the prime example of a PTP of which activating as well as inactivating mutations are associated with human disease. In addition, allelic variants of *PTPN21, PTPRA, PTPRD, PTPRJ, PTPRO* and *PTPRZ1* have been linked to human diseases, but the underlying mechanism remains to be elucidated. Intriguingly, the DUSP genes that are mutated in the germline and are responsible for hereditary human diseases encode enzymes, including phosphatase and tensin homolog (PTEN), myotubular myopathy-related DUSPs and laforin, which primarily act on nonproteinaceous substrates such as phospholipids [13, 14] and phosphorylated carbohydrates [15]. In all these cases, the hereditary alteration results in total or partial loss of function of the affected DUSP (Table 1). Finally, inactivating mutations in two class-IV eyes absent Asp-based PTP genes are causative in developmental disorders. The spectrum of mutations in PTP genes and their effects on catalytic activity warrant the development of specific PTP-inhibiting or PTP-activating therapies, respectively.

Animal models to study PTP physiology

Previously [4] we highlighted that knowledge concerning the physiological roles of individual classical PTPs is largely derived from studies with – often loss-of-function – mutant mouse models. Since that time several new mouse models have appeared in the literature and – in line with our prediction – compound studies in knockout mice have also been undertaken. Here, we will briefly update the classical PTP mouse knockout catalog. Subsequently, we will highlight the impact of the application of zebrafish as an animal model system on our knowledge of PTP functioning during early development.

Mouse models: an update

Around 2007 already a comforting number of classical PTP genes had been knocked out in the mouse genome [4], leaving work to be completed for some nine additional genes. Unfortunately, for genes *Ptpn18, Ptpn20, Ptpn21* and *Ptpru*, mouse models are still not available. The International Knock-out Mouse Consortium (www.knockoutmouse.org) has vectors prepared for *Ptpn18*, and targeted ES cells are ready for *Ptpn20, Ptpn21* and *Ptpru*, so a first hurdle has been taken and the waiting is for research funds and brave, interested scientists to take up the remaining challenges.

The mouse genome encodes many PTPs that are structurally related, and many PTP knockout mice

Table 1. PTP genes involved in hereditary human diseases. DEP1, density-enhanced phosphatase; EYA, eyes absent; GLEPP1, glomerular epithelial protein 1; MTM(R), myotubularin(-related); OMIM, Online Mendelian Inheritance in Man.

Gene	Protein	Chromosome	Disease	OMIM	Mutation output
Cys-based classical PTPs					
PTPN1	PTP1B	20q13.1-13.2	Noninsulin-dependent diabetes mellitus	125853[a]	Gain-of-function[b]
PTPN2	TCPTP	18p11.3-11.2	T-cell leukemia, homeobox 1	186770[c]	Loss-of-function
			Inflammatory bowel disease 21	612354[d]	Locus SNP[b]
			Rheumatoid arthritis		Locus SNP[b]
			Insulin-dependent diabetes mellitus	222100[d]	Locus SNP[b]
PTPN11	SHP2	12q24	Noonan syndrome	163950[a]	Gain-of-function
			Leopard syndrome	151100[a]	Loss-of-function
			Metachondromatosis	156250[a]	(incomplete penetrance) Gain-of-function
			Juvenile myelomonocytic leukemia (cardiofaciocutaneous syndrome)	607785[a] 115150[a]	(oogenesis and twinning)
PTPN14	PEZ	1q32.2	Congenital lymphedema	613611[a]	Loss-of-function
PTPN21	PTPD1	14q31.3	Schizophrenia	181500[a]	Nonsynonym. markers[b]
PTPN22	LYP	1p13.2	Insulin-dependent diabetes mellitus	222100[d]	Gain-of-function and promoter SNP[b]
			Rheumatoid arthritis	180300[a]	Gain-of-function[b]
			Systemic lupus erythematosus	152700[a]	Gain-of-function[b]
			Hashimoto thyroiditis	140300[d]	Gain-of-function[b]
			Graves thyroiditis	275000[d]	Gain-of-function[b]
			Familial hypoadrenocorticism (Addison's disease)	240200[d]	Gain-of-function[b]
			Psoriasis	177900[a]	Locus SNP[b]
PTPRA	RPTPα	20p13	Schizophrenia	181500[a]	SNP[b]
PTPRC	CD45	1q31-q32	Severe combined immunodeficiency	608971[a]	Loss-of-function
			Multiple sclerosis	126200[a]	Splicing alteration[b]
			Hepatitis C virus susceptibility	609532[a]	Splicing alteration[b]
PTPRD	RPTPδ	9p24.3-p23	Restless legs syndrome	610438[d]	2 intragenic SNPs[b]
PTPRJ	DEP1	11p11.2	Early-onset familial colorectal cancer	114500[a]	Loss-of-function
PTPRO	GLEPP1	12p12.3	Idiopathic nephrotic syndrome	614196[a]	Loss-of-function
PTPRQ	PTPS31	12q21.2	Autosomal-recessive nonsyndromic hearing loss 84 (DFNB84)	613391[a]	Loss-of-function
PTPRZ1	RPTPζ	7q31.3	Schizophrenia	181500[a]	SNP[b]
Cys-based DUSP PTPs					
PTEN	PTEN	10q23.3	PTEN hamartoma tumor syndrome	601728[c]	Loss-of-function
			Macrocephaly/autism syndrome	605309[a]	Loss-of-function
MTM1	MTM1	Xq28	X-linked myotubular myopathy (XLMTM)	310400[a]	Loss-of-function
MTMR2	MTMR2	11q22	Charcot–Marie–Tooth disease 4B1	601382[a]	Loss-of-function
SBF2	MTMR13	11p15.4	Charcot–Marie–Tooth disease 4B2	604563[a]	Loss-of-function
MTMR14	MTMR14	3p25.3	Modifier of autosomal centronuclear myopathy	160150[a]	Loss-of-function
EPM2A	Laforin	6q24	Myoclonic epilepsy of lafora	254780[a]	Loss-of-function
Asp-based PTPs					
EYA1	EYA1	8q13.3	Branchio-oto-renal syndrome 1	113650[a]	Loss-of-function
			Oto-facio-cervical Syndrome	166780[a]	Loss-of-function
EYA4	EYA4	6q23	Autosomal-dominant deafness 10	601316[a]	Loss-of-function
			Dilated cardiomyopathy 1J	605362[a]	Loss-of-function

[a] Phenotype description, molecular basis known. [b] Allelic variants. [c] Gene with known sequence and phenotype. [d] Mendelian phenotype or locus, molecular basis unknown.

display only mild defects, suggesting redundancy between PTPs. Compound knockout mouse models lacking multiple PTPs are starting to emerge, such as the type R2A subfamily of receptor-type PTPs (RPTPs) (discussed later). An elegant recent study describes neuronal cell-specific double knock out of PTP1B and TCPTP, which revealed additive effects of PTP1B and TCPTP in the prevention of diet-induced obesity [16].

Ptpn4

On the basis of cell model experiments, the 4.1 protein/ezrin/radixin/moesin (FERM) and PSD95/disc large/zona occludens protein (PDZ) domain-containing PTPN4/megakaryocyte PTP/PTP-MEG1 has been implicated in the modulation of T-cell-receptor signaling. However, *Ptpn4* knockout animals showed normal T-cell development and T-cell-receptor (TCR) signaling, suggesting that the function of PTPN4 may be replaced by other PTPs [17]. Knockout animals were available for two additional genes (*Ptpn3* and *Ptpn13*) that encode FERM- and PDZ-bearing PTPs, and so Bauler and colleagues created compound deficient animals for the non-receptor type 5 and 7 subclasses of cytosolic PTPs [18]. T cells from triple-mutant mice, however, still developed normally and showed wild-type cytokine-secretion and proliferative responses to TCR stimulation. Thus, less is not always more.

Ptpn5

Ptpn5 encodes striatal-enriched phosphatase (STEP) isoforms, and, together with *Ptpn7* and *Ptprr*, it forms the receptor type 7 subfamily of PTPs that are characterized by the presence of a kinase interaction motif that mediates association with several mitogen-activated protein (MAP) kinases [19]. As found in the knockout mouse models for *Ptpn7* [20] and *Ptprr* [21], *Ptpn5* mutant mice are viable and fertile, display no overt morphological abnormalities but do show mitogen-activated protein kinase (MAPK) hyperphosphorylation in relevant tissues compared with samples from wild-type mice [22]. STEP modulates synaptic strengthening not only via MAP kinases but also by dephosphorylating the PTK Fyn, the glutamate receptor 2 (GluR2) subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and the 2B subunit of the *N*-methyl-D-aspartate receptor (NMDAR). The latter events trigger NMDAR and AMPAR internalization and thus have bearing for diseases characterized by cognitive deficits [23]. Indeed, recent work in an Alzheimer's disease mouse model revealed that reduction of STEP levels at least partially reverted the cognitive and cellular deficits in these mice [24]. Whether STEP inhibitors have therapeutic potential in humans remains to be tested.

Ptpn23

Ptpn23 encodes an intriguing member of the PTP family, His-domain containing PTP (HD-PTP), for which tumor-suppressive potential has been reported but catalytic activity is controversial. HD-PTP was shown not to harbor any phosphatase activity because of an amino-acid divergence of a key residue in the PTP domain [25]. In contrast, HD-PTP activates Src by direct dephosphorylation of the inhibitory C-terminal phosphorylation site in Src [26]. HD-PTP fulfils an essential role during mouse development and in adult epithelial cells of many organs because insertion of a β-galactosidase-containing gene trap in the *Ptpn23* gene proved to be homozygously lethal around embryonic day 9.5 [27]. Much more work is needed to establish the underlying mechanism.

Ptprh

Ptprh encodes stomach cancer-associated PTP-1 (SAP-1), an R3 subgroup receptor-type PTP with a single cytoplasmic catalytic domain and multiple fibronectin type III (FNIII)-like domains extracellularly. Expression of SAP-1 in mice is mainly restricted to the digestive tract and the protein localizes to the microvilli-bearing brush border of gastrointestinal epithelial cells. Remarkably, SAP-1-deficient mice did not show any changes in intestinal epithelium morphology but, when crossed onto an adenomatous polyposis coli intestinal tumor background, a marked inhibition of tumorigenesis was obtained [28]. The challenge now is to unravel how the microvillus-specific SAP-1 regulates intestinal tumor formation, and first hints point at Src family tyrosine kinases (SFKs) and the Src homology type 2 (SH2) domain-containing proteins Grb2 and Fyn that, in complex with SAP-1, regulate cell morphology and motility [29].

Ptprt

The R2B RPTPρ is the most frequently mutated classical PTP in human cancer [30]. Like RPTPμ it facilitates homophilic cell-to-cell aggregation via transdimerization of its extracellular region that consists of cell adhesion molecule-like motifs, including a MAM (meprin/A5 protein/RPTPmu) domain and an immunoglobulin domain and four FNIII repeats. Tumor-derived mutations impair cell-to-cell aggregation, thereby facilitating cancer progression [7]. In mice, RPTPρ is encoded by *Ptprt* and is expressed in the CNS where it was shown to regulate synapse formation through interactions with neuroligins and Fyn. Intriguingly, Fyn could inhibit RPTPρ activity by phosphorylating Y912 in the PTP domain, which subsequently reinforced RPTPρ homophilic interactions and prevented its heterophilic association with neuroligins [31]. Studies exploiting *Ptprt*-deficient animals allowed the identification of the

submembranous cytoskeleton protein paxillin as a direct RPTPρ substrate in colon [32]. Importantly, these *Ptprt* knockout mice appear to be highly susceptible to chemical induction of colon cancers, firmly establishing *PTPRT* as a tumor-suppressor gene.

Ptprc and *Ptprj*

The receptor-type PTPs CD45 and CD148 are both critical regulators of SFKs in hematopoietic cells, and knockout mice for the individual genes have been studied [4]. In view of the central role that SFKs play in immune-cell function and disease, Weiss' group investigated possible overlap of CD45 and CD148 functions in the immune system exploiting these *Ptprc* and *Ptprj* mutant animals [33]. Much like CD45, CD148 proved a positive regulator in B cells and macrophages. Combined CD148/CD45 deficiency led to aberrancies in B- and myeloid-lineage development and to defective immunoreceptor signaling as a result of hyperphosphorylation of the inhibitory tyrosine site in the C terminus of SFKs [33]. Thus, the SFK inhibitory site is a common substrate for both PTPs and this implies an unexpected level of redundancy in B and myeloid lineages. Given that *Ptpn22* also encodes a hematopoietic PTP that can regulate SFKs lymphoid tyrosine phosphatase (LYP)/PEST domain-enriched tyrosine phosphatase (PEP), further clues on the signaling networks in hematopoietic cells may require reassessment of the actions of SFK in B and myeloid lineages in additional compound mutant animals.

Ptprd Ptprf and *Ptprs*

Knockout mice have been established for the three genes that encode the R2A subfamily RPTPs leukocyte common antigen related (LAR), RPTPδ and RPTPσ [4]. RPTPδ/RPTPσ knockout intercrosses have also been studied and resulted in animals that were paralyzed and could not breathe as a result of extensive muscle dysgenesis and spinal cord motoneuron loss, demonstrating functional redundancy within the subfamily [34]. Meanwhile, the phenotype of mice having a combined deficiency for LAR and RPTPσ has also been described. Double-knockout embryos displayed severe urogenital malformations and craniofacial defects, providing a picture in which apoptosis-mediated tissue morphogenesis is controlled by opposing actions of the PTK Ret and R2A PTPs [35]. Unfortunately, the LAR and RPTPδ genes (*Ptprf* and *Ptprd*, respectively) reside on the same chromosome, making such a double knock out rather laborious but certainly interesting because both proteins have a role in synaptic plasticity and memory. Mice lacking all three R2A PTPs will probably be 'early embryonic lethal'.

Zebrafish as a model to study PTPs during development

The zebrafish is an excellent model system that facilitates the study of gene function and is increasingly being used to model human diseases [36]. Zebrafish embryos are ideally suited for experimentation because it is easy to obtain large numbers of embryos (each adult zebrafish pair produces one to two clutches per week of 100–200 embryos per clutch). The embryos are easily accessible for experimentation because they develop outside the mother, and the embryos are transparent, allowing the experimentator to easily follow embryonic development. In addition, transgenesis is feasible and embryonic development is rapid, which makes the zebrafish embryo ideally suited for intravital imaging. An ever-increasing number of transgenic lines expressing fluorescent marker proteins under the control of specific promoters are available, which allow time-lapse (confocal) imaging of organs, tissues or cells of interest while they develop. Chemically induced mutagenesis is feasible in zebrafish and large-scale forward genetic screens have yielded a wealth of genetic mutants that were selected based on particular phenotypes [37, 38]. Target selected gene inactivation allows for the selection of genetic mutants that carry mutations in specific genes [39], and zinc finger technology has also been developed to inactivate target genes [40, 41]. Transient target protein knockdown can be achieved by microinjection of morpholinos [42, 43]. Finally, administration of chemical compounds to zebrafish embryos is easy by simple addition to the aqueous embryo medium, and, as a result, large numbers of compounds can easily be screened using (aspects of) zebrafish development as read-out [44]. Taken together, the zebrafish is an ideal model system for analysis of gene function at the genetic, molecular and cellular levels in whole organisms.

Zebrafish PTP

In the zebrafish genome, 48 classical PTPs were identified [45] (Fig. 1). Interestingly, comparison of PTP sequences in five distinct fish genomes led to the surprising discovery that Ptpn20 encodes a large PTP with multiple functional domains, closely resembling PTP-BAS like (PTP-BL), rather than a small protein encoding little more than a PTP domain. In fact, the human *PTPN20* gene has a similar structure to that of zebrafish *ptpn20*, which was confirmed by reverse

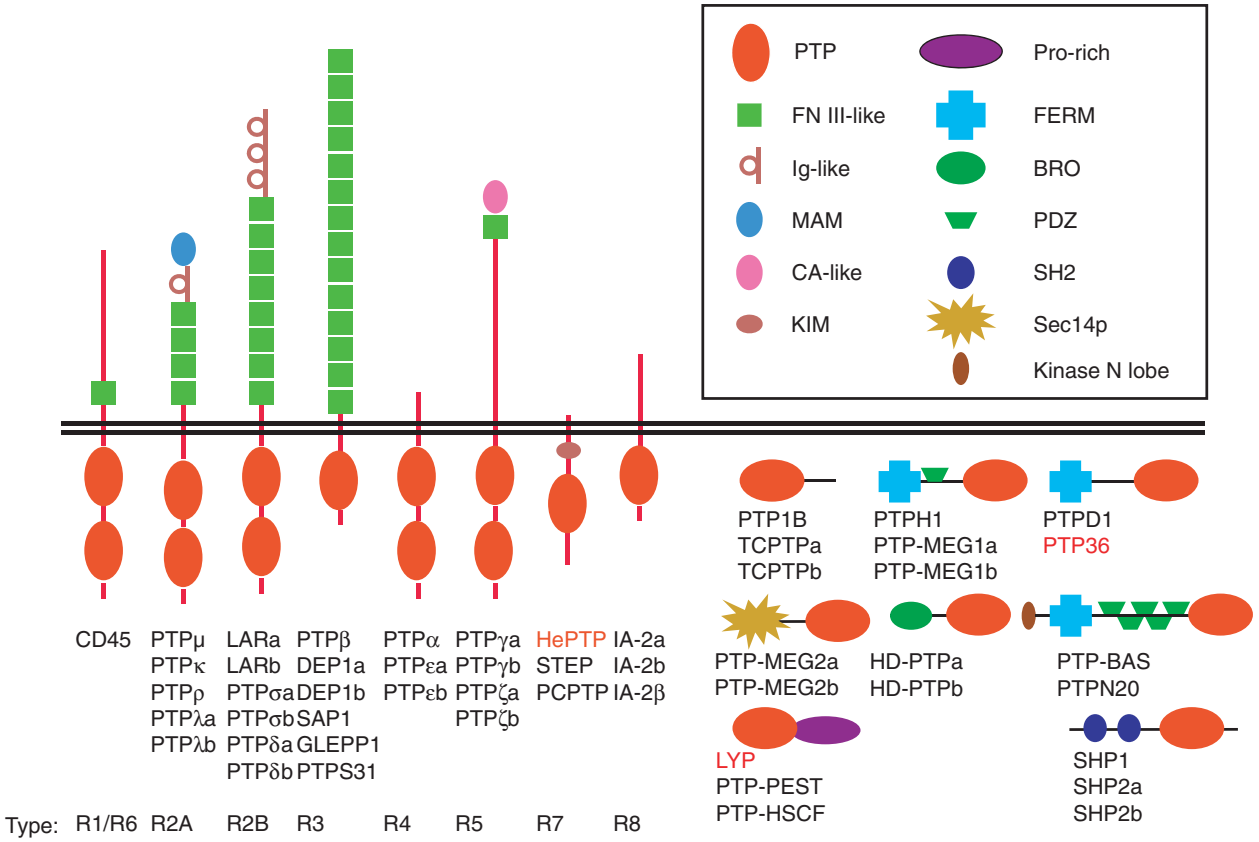

Fig. 1. The classical PTP family in zebrafish. Forty-eight classical PTPs have been identified in zebrafish and are depicted schematically. Homologues of all but three of the human PTPs have been identified in zebrafish. The ones that were not identified in zebrafish are indicated in red (HePTP, LYP and PTP36). Many PTPs are duplicated in the zebrafish genome, indicated as 'a' and 'b' (see the text for details). Note that PTPN20, which was reported to encode little more than a PTP domain, is actually an ohnolog of PTP-BAS with a KIND domain, a FERM domain, five PDZ domains and a PTP domain in zebrafish as well as in humans. The schematic representation of the different functional domains is indicated in the figure key (top right). BRO, BROMO domain; CA, carbonic anhydrase; Ig, immunoglobulin; KIM, kinase interaction motif; MAM, meprin/A5 protein/RPTPmu domain; Sec14p, lipid-binding domain.

transcription PCR [46]. All but three mammalian PTP genes are represented in the zebrafish genome. The missing PTPs may have been lost in evolution or perhaps are located in poorly sequenced areas of the genome. Fourteen PTP genes are duplicated in the zebrafish genome as the result of genome duplication in teleosts that occurred 320 million years ago [47, 48]. We have established the expression patterns of all PTP genes in zebrafish and found that expression of half of the duplicated genes is completely overlapping, whereas the other half has distinct, or even mutually exclusive, expression patterns [45]. The duplication-degeneration-complementation model [49] suggests that degenerative mutations in regulatory elements increase the probability of duplicate gene preservation. If only random mutations in genes had been considered, most duplicated genes would have disappeared by now. PTP function has been studied in zebrafish by transient, morpholino-mediated knockdown, in genetic loss-of-function mutants and by expression of exogenous PTP genes and mutants thereof. Morpholino-mediated knockdowns provide rapid and efficient means for assessing the function of PTPs in zebrafish embryogenesis.

PTPs in zebrafish gastrulation

We and others have found that several PTPs have an essential role in cell movements during gastrulation. In particular, convergence and extension cell movements that normally form the medio-lateral body axis [50] are affected by modulation of PTP expression. For instance, morpholino-mediated knockdown of SH2 domain-containing PTP 2 (Shp2) resulted in defective gastrulation cell movements, whereas cell fate specification was not affected [51]. Interestingly, Shp2 acted

upstream of the SFKs, Fyn and Yes, and the small GTPase, RhoA, because expression of Fyn, Yes or RhoA rescued the Shp2 knockdown-induced defects. RPTPα and PTPε are well-known activators of SFKs and they are also required for normal gastrulation cell movements in an SFK- and RhoA-dependent manner [52]. Not only transient morpholino-mediated knockdown of RPTPα, but also a genetic mutant with a nonsense mutation to the N-terminal side of the transmembrane domain of RPTPα displayed convergence and extension cell-movement defects. Moreover, RPTPψ, the ortholog of human RPTPλ, which is encoded by ptprua, is required for normal segmentation of zebrafish embryos. RPTPψ knockdown does not affect paraxial mesoderm specification, but RPTPψ knockdown results in defects in convergence and extension cell movements during gastrulation, illustrated by changes in marker gene expression that are typical for convergence and extension cell movement defects [53]. It remains to be determined whether SFKs and RhoA act downstream of RPTPψ. Recent studies indicated that knockdown of PTP-BL (*ptpn13*) or Ptpn20 led to gastrulation cell movement defects. Interestingly, these knockdowns were rescued by dominant negative RhoA, while RPTPα and PTPε knockdowns were rescued by constitutively active RhoA. A model is emerging in which PTP-mediated modulation of RhoA activity results in proper cell polarization, which is at the basis of normal convergence and extension cell movements during gastrulation [46].

Modeling human syndromes in zebrafish

SHP2 is associated with Noonan and LEOPARD syndromes in humans [54–56] (Table 1). Fifty per cent of patients with Noonan syndrome have activating mutations in SHP2, and LEOPARD syndrome is often associated with inactivating mutations in SHP2. Introduction of the corresponding mutations in zebrafish Shp2 leads to activation and inactivation of Shp2 catalytic activity, respectively. Moreover, expression of mutant Shp2 in zebrafish embryos by microinjection of synthetic RNA at the one-cell stage induces developmental defects as early as gastrulation. Strikingly, the developmental defects at later stages in zebrafish embryos are highly reminiscent of the developmental defects in humans, including short stature (reduced embryonic body axis extension), hypertelorism (eyes set wider apart and other craniofacial defects) and cardiac defects (in particular cardiac edema) [51]. Interestingly, comparison of the effects of Shp2 knockdown with the effects of expression of catalytically inactive LEOPARD mutant Shp2 indicated that certain aspects of LEOPARD syndrome pathogenesis can be explained by combined loss of Shp2 catalytic function and retention of a phosphatase-independent function that is mediated through its SH2 domains [57].

Several other PTPs have essential functions in zebrafish embryonic development. Knockdown of PTP/ezrin-like (Pez) (*ptpn14*) results in defects in the development of several organs that normally express Pez, including the heart, resulting in cardiac edemas, impaired looping of the heart and defective valvogenesis, causing blood regurgitation and impaired blood flow. In addition, the somite boundaries are irregular, the ventricular zone is expanded and the pharyngeal arches are malformed, which is accompanied by enhanced cell densities in these structures. Pez knockdown results in loss of expression of transforming growth factor beta 3 (TGF-β3), which, combined with results in tissue-culture cells, indicates that Pez is a crucial regulator of TGF-β3 signaling and suggests that the Pez knockdown-induced defects are at least partially mediated by impaired TGF-β3 signaling-dependent epithelial to mesenchymal transition [58].

Knockdown of PTP? results in the accumulation of synaptic vesicles in the axon terminals of olfactory sensory neurons [59]. This effect was rescued by expression of PTP?. Interestingly, expression of a catalytically inactive mutant of PTP? in olfactory sensory neurons also enhances the accumulation of synaptic vesicles. These results suggest that PTP? controls the number of olfactory sensory neuron-mitral cell synapses by suppressing their excessive increase. These results are consistent with an essential role for the type 2a RPTPs in synaptogenesis (discussed later).

PTP-based cancer model

A prominent member of the PTP superfamily is PTEN, one of the most frequently mutated tumor suppressors in human tumors. The zebrafish genome encodes two *pten* genes – *ptena* and *ptenb* – and Ptena and Ptenb exhibit similar lipid phosphatase activity. Based on sequence conservation and catalytic activity, both Ptena and Ptenb are considered to be orthologs of human PTEN [60]. Genetic mutants with nonsense mutations well upstream of the catalytic site of both Ptena and Ptenb have been identified. Homozygous $ptena^{-/-}$ or $ptenb^{-/-}$ zebrafish are viable and fertile and do not display developmental defects, which is consistent with the notion that both zebrafish pten genes are functional. Interestingly, $ptena^{+/-}$ $ptenb^{-/-}$ and $ptena^{-/-}$ $ptenb^{+/-}$ zebrafish develop tumors that were characterized pathologically as hemangiosarcomas and these tumors stain positive for endothelial

cell markers, consistent with the diagnosis [61]. Moreover, immunohistochemistry indicated that the tumors contain rapidly dividing cells (PCNA-positive) as well as elevated pAkt and pGSK3 levels, consistent with the loss of Pten. Zebrafish embryos that lack all functional Pten (*ptena*$^{-/-}$ *ptenb*$^{-/-}$) are embryonic lethal, which is consistent with other animal models that lack Pten. The *ptena*$^{-/-}$ *ptenb*$^{-/-}$ embryos display various hyperplastic/dysplastic defects [60]. Zebrafish embryos that retain a single wild-type pten allele do not show developmental defects whatsoever, indicating that Ptena and Ptenb have redundant functions in development.

PTPs in health and disease: some recent advances

From the above it is clear that deciphering the molecular basis of hereditary diseases and the generation and study of genetically modified animals has added greatly to our knowledge of PTP physiology. Notably, the animal model systems will be complemented in the near future with induced pluripotent stem cells derived from affected individuals to help assess the function of PTPs. Also, intravital imaging in zebrafish will prove a useful tool with which to unravel disease mechanisms at the molecular-genetic and cellular levels and in a developmental context. To illustrate the seminal contribution of studies in cell and animal models and on patient material we will briefly review some research fields that experienced great progress over the past few years as a result of these approaches: the role of PTPs in bone morphogenesis; the impact of PTPs on neuronal and neuromuscular synaptogenesis; and, finally, the connection of PTPs with CNS diseases.

PTPs and bone development

Bones provide physical support for the organism, serve as a reservoir for calcium and several other important minerals, and provide a location where immune cells and other cell types can proliferate and function. Once formed, bone matrix is maintained by the opposing activities of mesenchyme-derived osteoblasts and osteoclasts that are produced from hematopoietic precursors [62–64]. Both cell types function in close proximity, leading to continuous synthesis and degradation of bone matrix that is critical for maintaining its amount and proper physical properties. Disruption of bone turnover is a significant cause or contributing

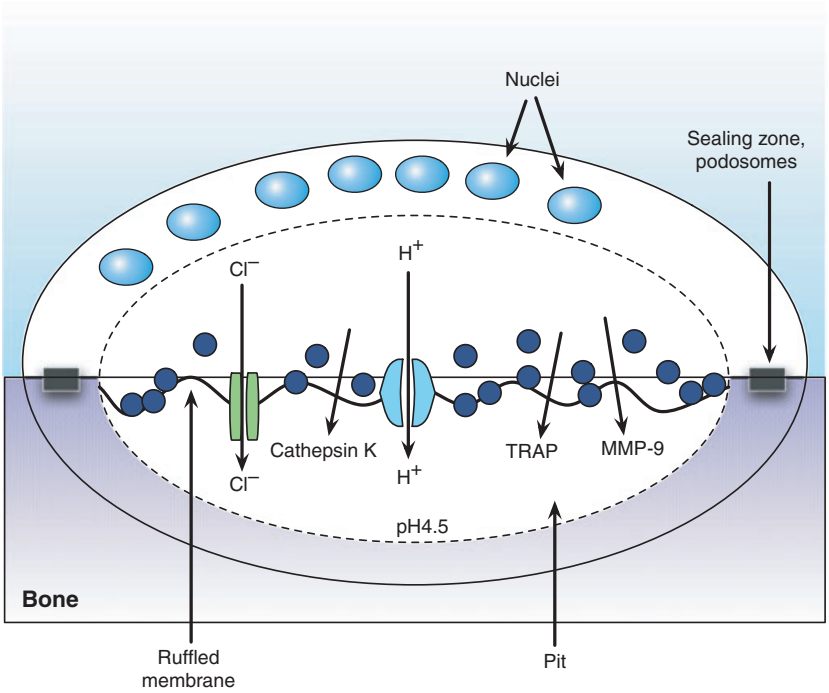

Fig. 2. Schematic drawing of a cross-section of a bone-resorbing osteoclast. Osteoclasts are very large, multinucleated cells that attach to the underlying bone matrix using podosomes, thereby isolating the bone underneath them. Osteoclasts secrete onto this bone a mixture of proteolytic enzymes (via vesicles that fuse with the cell's ventral membranes, and acid, using various channels and transporters. The proteases and acid degrade the organic and inorganic components of the bone matrix and form a pit in the bone surface. MMP-9, matrix metalloproteinase 9; TRAP, tartrate-resistant acid phosphate.

factor in osteoporosis and other types of bone disease [64,65].

Osteoclasts are formed by fusion of monocyte-macrophage precursor cells in response to molecular signals they receive from their environment. Two key molecules in this respect are receptor activator of nuclear factor kappaB ligand (RANKL) and colony stimulating factor 1 (macrophage) (M-CSF/CSF-1); the presence of both molecules is sufficient to drive differentiation of precursor cells into mature osteoclasts *in vitro* [66]. Osteoclasts attach firmly to bone matrix using specialized adhesion structures called podosomes (Fig. 2). In bone-resorbing osteoclasts, podosomes are arranged in a large ring at the cell periphery, referred to as the sealing zone in cells grown on mineralized matrix. The cell then secretes, onto the bone surface, hydrochloric acid and proteases that degrade the organic and inorganic components of the bone matrix. Secretion occurs by fusion of secretory vesicles containing proteases with the ventral plasma membrane of osteoclasts (the 'ruffled membrane') and by activation of ion channels located in this membrane [66].

Tyrosine phosphorylation of proteins is critical for osteoclast production and function, as illustrated by the pivotal role of the Src PTK in these cells. Genetic or pharmacologic inhibition of Src in mice or in cultured osteoclasts reduces the ability of these cells to resorb bone and to organize their podosomal adhesion structures properly [67–69]. Phosphorylation also plays key roles in signaling mediated by RANKL and by M-CSF, whose receptor is the PTK c-Fms [70, 71]. Pyk2, the product of the PTK2b gene, has also been shown to play an important role in osteoclasts [72] and in osteoblasts [73]. Activation of integrin signaling by physical contact of osteoclasts with matrix significantly increases protein phosphorylation in or around podosomes, rendering them among the most highly phosphorylated structures in osteoclasts [74]. As tyrosine phosphorylation is regulated by the opposing activities of PTKs and PTPs, PTPs play central roles in this cell type. Accordingly, several PTPs (discussed below) have been shown to regulate osteoclast production and/or activity.

PTP Epsilon

The nonreceptor isoform of PTPε [cytosolic (cyt)-PTPε] is expressed in osteoclasts; osteoblasts do not express any form of this PTP [75]. Homozygous PTPε knockout (EKO) mice exhibit increased trabecular bone mass; this is most prevalent in young female mice [75]. This phenotype is caused by reduced osteoclast function, which is secondary to reduced ability of EKO osteoclasts to adhere to bone *in vivo*. Affected mice also exhibit poor recruitment of hematopoietic precursor cells from the bone marrow to the circulation, a process that depends on proper osteoclast activity [76]. Further studies showed that the structure, organization and stability of podosomes in EKO osteoclasts are abnormal, indicating that cyt-PTPε participates in regulating podosome function [75, 77]. At the molecular level, cyt-PTPε links activated integrins with the downstream PTK, Src. Upon activation of integrin molecules, cyt-PTPε undergoes C-terminal phosphorylation at Y638 by a partially active Src; this directs the phosphatase to dephosphorylate Src at its inhibitory Y527, thus fully activating the kinase. cyt-PTPε thus functions as a positive-feedback regulator and maximizes the activity of Src downstream of activated integrins [77].

MKP-1

Female Mkp-1 knockout (MKO) mice exhibit mildly reduced bone mass and fewer osteoclasts than do wild-type controls [78]. Ovariectomy induces a similar amount of bone loss in MKO mice and control mice, suggesting that Mkp-1-deficient osteoclasts are more active. In agreement, spleen-derived macrophages lacking Mkp-1 yielded fewer osteoclasts when cultured with RANKL, but these cells were more active than the same cells from controls in resorbing matrix. Increased bone resorption was noted also when lipopolysaccharide was injected into the calvarial periosteum [78] and palate [79] of MKO mice. The activities of p38 MAPK and c-Jun N-terminal kinase (JNK), but not of extracellular-signal-regulated kinase, were increased in MKO osteoclasts following stimulation with RANKL. While Mkp-1 probably acts in osteoclasts by regulating MAP kinases, the precise molecular processes that this phosphatase controls remain to be determined.

SHP1

Mice carrying the *motheaten* mutation that inactivates Shp1 exhibit reduced bone mass and increased numbers and activity of osteoclasts, indicating that Shp1 inhibits osteoclast formation and function [80, 81]. Similar conclusions were reached when *motheaten* osteoclasts were produced and analyzed *in vitro* [80, 81]. Further studies of SHP1 in osteoclasts and in relevant model cell lines suggest that SHP1 is a negative regulator of osteoclastogenic signaling downstream of RANKL along the receptor activator of nuclear factor

kappaB/tumor necrosis factor receptor associated factor 6 pathway [82].

SHP2

Shp2 knockout mice die during gestation, necessitating the use of conditional deletion models to study the role of this PTP *in vivo*. Accordingly, mice in which Shp2 is deleted in tissues that express Cre recombinase under the direction of a tamoxifen-inducible estrogen receptor displayed severe alterations in cartilage structure and significant increases in trabecular bone mass, along with almost total absence of osteoclasts [83]. The ability of bone marrow cells to differentiate into osteoclasts *in vitro* in response to M-CSF and RANKL was significantly reduced in these mice, correlating with reduced activation of Akt/protein kinase B (PKB) in bone marrow cells following their stimulation with M-CSF [83]. Shp2 then performs a critical role in support of osteoclastogenesis *in vivo*, a role that is the opposite of the closely related Shp1.

PTPRO/PTP-oc

PTP-oc (also known as PTP-Φ or PTPROt) is an RPTP that is produced by the proximal promoter of the *PTPRO* gene [84]. Studies in cell culture indicate that PTP-oc might positively regulate osteoclast production and activity. Inhibition of PTP-oc expression in rabbit osteoclasts reduced their activity [85], while overexpression of this PTP in U937 monocytic cells promoted their differentiation into osteoclast-like cells and increased their bone-resorbing activity [86]. Reduced expression of PTP-oc correlated with hyperphosphorylation of Src at its inhibitory Y527, while overexpression of PTP-oc increased Src activity [85, 86]. Collectively, these and other results suggest that PTP-oc dephosphorylates and activates Src in osteoclasts; the downstream effects of this activity may be mediated through activation of nuclear factor of kappa light polypeptide gene enhancer in B-cells and JNK2 [87] and by the RANKL pathway. Finally, young male mice expressing transgenic PTP-oc exhibited increased bone resorption and bone loss [88]. These intriguing results would be supplemented significantly by studies of bone structure in a PTP-oc knockout mouse model.

PTP-PEST

The ubiquitous nonreceptor protein tyrosine phosphatase, containing proline/glutamate/serine/threonine-rich domains, PTP-PEST, inhibits cell spreading and membranal protrusion, and promotes focal adhesion turnover and cell migration [89]. PTP-PEST binds paxillin and controls membrane protrusion by inhibiting Rac1 and activating RhoA [90]. PTP-PEST also interacts with the proline serine threonine-rich phosphatase interacting protein (PSTPIP), which is both a substrate of PTP-PEST and a scaffold that provides this PTP with access to the Wiskott-Aldrich syndrome protein (WASP) during actin remodeling [91, 92]. The adhesion-related Crk-associated protein (p130CAS) [93] and Pyk2 [94] are also substrates of PTP-PEST. It is therefore not surprising that PTP-PEST also helps to regulate osteoclast adhesion and differentiation. PTP-PEST localizes to the podosome structures and peripheral sealing zone of resorbing osteoclasts, physically associates with leupaxin, gelsolin, WASP, Src, PYK2 and PSTPIP [95–97], and activates Src [98]. Overexpression of PTP-PEST induced Src activation and phosphorylation of cortactin and WASP; in parallel, the number of osteoclasts that displayed an intact sealing zone-like structure, which is typical of functional osteoclasts, also increased [98].

CD45

The RPTP CD45 is found on the surface of various hematopoietic cells, including osteoclasts. Lack of CD45 activates Src and downstream signaling events, suggesting that this PTP inhibits Src [99]. CD45-deficient bone marrow cells produce fewer osteoclasts when cultured *in vitro* with RANKL, suggesting that CD45 mediates the critical role of RANKL in differentiating precursors into mature osteoclasts. Treatment of CD45-deficient bone marrow cells with the Src inhibitor PP2 corrects this defect, indicating that CD45 affects RANKL signaling by inhibiting Src. In agreement, RANKL failed to increase the development of osteoclasts in bones of CD45 knockout mice; this finding correlated with increased bone mass and reduced osteoclast-dependent recruitment of hematopoietic progenitor cells from the bone marrow into the circulation of these mice [76, 99].

Tyrosine phosphorylation of proteins in osteoclasts is central in the production of these cells and for their ability to resorb bone. Increasing awareness of the importance of the roles of PTPs in these processes has resulted in recent demonstration of the importance of several discrete PTPs in osteoclasts. Individual PTPs play specific roles in osteoclasts – some support, while others inhibit, the production or activity of osteoclast cells. Knowledge of the roles of individual PTPs is then required in order to identify good targets for

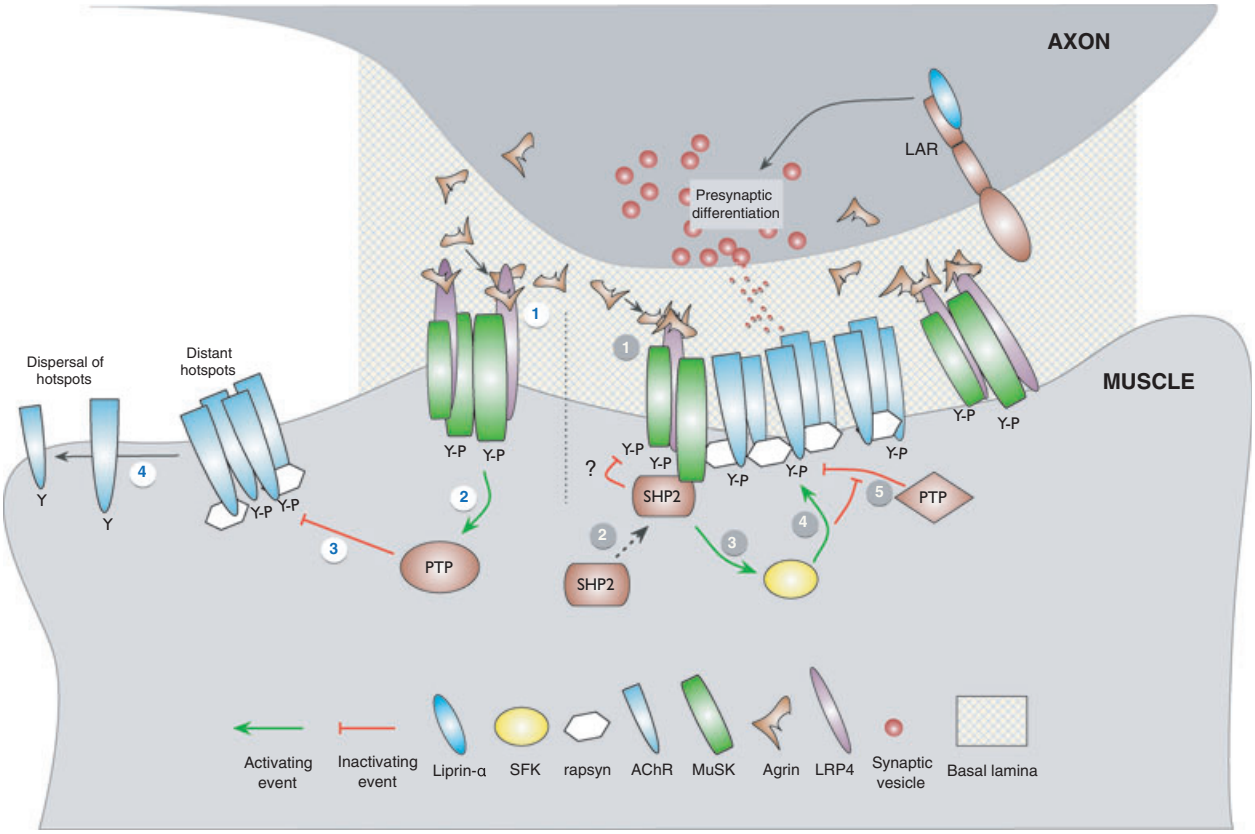

Fig. 3. The neuromuscular junction. Schematic showing some of the key components of a generic neuromuscular junction, highlighting the roles played by PTP enzymes (in brown). In the process depicted by grey numbered circles, activation of MuSK after agrin binding to low density lipoprotein receptor related protein 4 (LRP4), leads to recruitment of SHP2 to the complex (2), activation of SFK (3) and phosphorylation of AChR (4). This may also inhibit PTPs that normally dephosphorylate AChRs (step 5), and SHP2 may also be involved in the negative feedback of MuSK. In the process depicted by white numbered circles, activation of MuSK (1) can also activate uncharacterized PTPs (2) that then dephosphorylate more distant AChR hotspots (3), causing their dispersal (4). LAR family receptor-type PTPs are also implicated in presynaptic differentiation at the NMJ, requiring interactions with the protein liprin-α. Y, tyrosine; Y-P, phosphotyrosine. See the main text for more details.

inhibitors that may be used for therapeutic gain and, no less importantly, to know which PTPs not to target. Identifying all PTPs expressed in these cells is critical, as is characterizing the roles of specific PTPs in bone using knockout mice.

PTPs and synatopgenesis

In the last decade, PTPs have been increasingly implicated in the control of neuronal synapse formation and function. To illustrate this we will focus on their proposed roles in the neuromuscular junction and in CNS synapses.

The neuromuscular junction

Immature neuromuscular junctions (NMJs) form as embryonic motor axons contact myotubes, are stabilized and then remodeled. Axon terminals secrete agrin, a heparan sulfate proteoglycan (HSPG), which binds the tyrosine kinase muscle specific kinase (MuSK) in the postsynaptic cleft, triggering clustering and stabilization of acetylcholine receptor (AChR) complexes [100] (Fig. 3). Protein tyrosine phosphorylation is central to NMJ formation and stability. Agrin drives MuSK autophosphorylation, then AChR tyrosine phosphorylation by SFKs stabilizes the NMJ [101]. The cytoplasmic phosphatase SHP2 may govern neuregulin-mediated control of AChR expression at the NMJ [102], and PTPs, including SHP2, are direct regulators of NMJ formation and stability (Fig. 4). Both pervanadate, a broad PTP inhibitor, and SHP2 loss-of-function can cause AChR hotspot formation without agrin in Xenopus myotubes and increase MuSK phosphorylation and agrin-independent AChR clusters in C2C12 cells [103]. Curiously, focal activa-

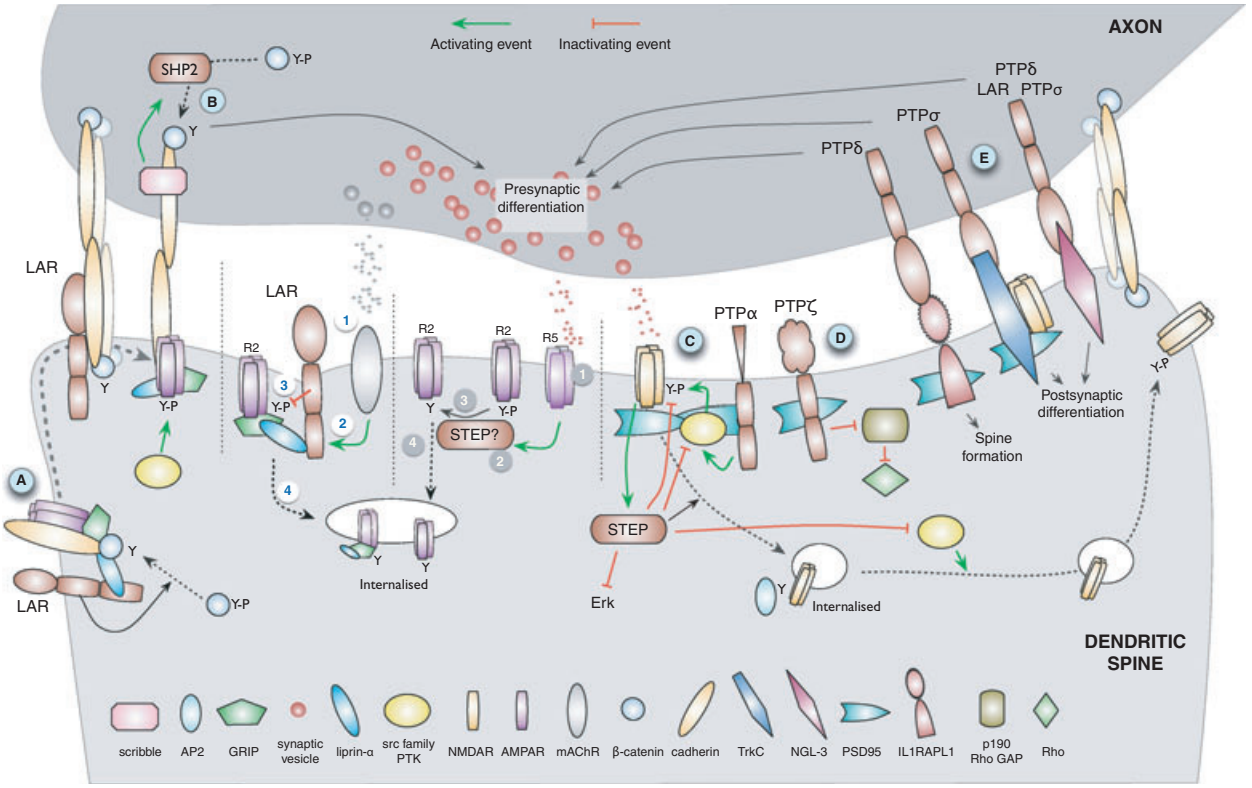

Fig. 4. The CNS excitatory synapse. Schematic showing a generic CNS synapse with a dendritic bouton contacted by an axon terminal. The suggested roles of several PTP enzymes (in brown) are shown. In process **A**, LAR recruits β-catenin through dephosphorylation, forming a complex of β-catenin/cadherin, liprin-α, GRIP and AMPAR. This facilitates the delivery of AMPARs and adhesion complexes into the synapse. In process **B**, cadherins/β-catenin adhesion complexes activate SHP2 presynaptically, possibly via scribble, leading to β-catenin dephosphorylation and ultimately to presynaptic differentiation. Process **C** depicts the activation of NMDAR, leading to stimulation of the PTP STEP. RPTPα can also activate local Src PTKs, causing phosphorylation and activation of NMDAR. Activation of STEP can potentially have several roles, in particular inactivating Src PTKs and NMDAR and inhibiting extracellular-signal-regulated kinase (Erk) signaling. Dephosphorylated NMDAR associates with adaptor protein 2 (AP2) and is internalized. Process **D** depicts inactivation of synaptic p190 RhoGAP by RPTPZ, relieving inhibition of Rho. Processes in **E** depict the *trans* interactions between type IIa RPTPs LAR, PTPσ and PTPδ, with ligands including TrkC, NGL-3 and IL1RAPL1, leading to spine formation, presynaptic maturation and postsynaptic differentiation. White circles depict activation of mAChR (1) followed by the association of LAR with AMPAR/GRIP/liprin-α complexes (2), dephosphorylation of AMPAR by LAR (3) and AMPAR internalization (4), contributing to LTD. The grey circles depict activation of AMPAR (1) causing activation of a PTP (2) (likely to be STEP) that dephosphorylates AMPAR (3), leading again to internalization (4). Dashed lines with arrowheads indicate translocation of proteins and complexes. Y, tyrosine; Y-P, phosphotyrosine See the main text for more details.

tion of MuSK reduces the phosphotyrosine content in distant AChR 'hotspots' and this is prevented by vanadate, implicating PTPs in hotspot dispersal. Loss of AChR clusters after agrin removal from myotubes is more rapid in myotubes lacking Src and Fyn PTKs [104]. This cluster loss is blocked by vanadate, again indicating PTP-dependence. However, treatment with vanadate does not affect spontaneous cluster formation without agrin [104]. SHP2 binding to MuSK is stimulated by agrin and depletion of SHP2 destabilizes AChR clusters. Although these are conflicting reports, in that loss of SHP2 leads to increased AChR clustering in one case, and to a decrease in the other, the studies used distinct cell systems. In addition, the context-specific roles of SHP2 could be quite distinct, for example as an inhibitor of MuSK, stimulator of Src PTKs or dephosphorylator of AChR.

In Drosophila, DLAR (an ortholog of the mammalian LAR family, type IIa RPTPs) functions in NMJ formation. DLAR controls body wall muscle innervation through interactions with the HSPGs syndecan (Sdc) and Dally-like (Dlp) [105]. Sdc and Dlp are high-affinity ligands for DLAR, but have a competing relationship during synaptogenesis. Sdc in motor axons regulates presynaptic bouton size and growth, where catalytically active DLAR is necessary. Dlp is in the

synaptic cleft matrix and can outcompete Sdc, inhibiting DLAR. This halts NMJ growth, allowing synapse maturation and control over neurotransmitter release. There is a fascinating link here with mammalian PTPσ (a DLAR relative), which binds both to HSPGs (including agrin) and to chondroitin sulfate proteoglycans, activating or inhibiting axon regeneration, respectively [106–108]. Although PTPσ has no defined role in NMJ formation, it does control diaphragm innervation alongside PTPδ [34].

CNS excitatory synapses

Excitatory synapses in the brain undergo synaptic plasticity, the cornerstone of learning and memory. There is growing interest in the functional regulation by PTPs (Fig. 4). In hippocampal excitatory synapses, LAR family RPTPs co-localize with post-synaptic density protein 95 (PSD95) postsynaptically. Catalytically inactive LAR and liprin-α-binding mutants of LAR support fewer dendritic spines and have smaller, miniature excitatory currents [109]. Disrupting interactions between liprin-α and glutamate receptor interacting protein (GRIP), a PDZ protein that assembles protein complexes with AMPARs, mirrors LAR/liprin-α disruption [109, 110]. AMPARs regulate fast excitatory synaptic transmission, and the AMPAR level in spines influences synaptic strength. Spine-associated AMPARs are greatly reduced upon disruption of LAR, suggesting that LAR regulates neurotransmitter receptor localization [109]. LAR might dephosphorylate β-catenin, driving β-catenin into spines and increasing its interactions with cadherins, GRIP/liprin-α and AMPARs, thus boosting synapse size and strength [109, 111] (Fig. 4). Liprin-α, GRIP, NR1 and PSD95 are probably not, however, LAR substrates [109]. RNA interference-driven loss of function of each of the three type IIa RPTPs (LAR, PTPσ and PTPδ) can reduce spine numbers and PSD95 co-localization, suggesting a complex, shared functionality.

Specific cell-to-cell adhesion is vital for synapse stabilization and signaling, with cadherins playing a central role [112]. Cadherin adhesion is regulated through cytoplasmic association with β-catenin and tyrosine phosphorylation of both proteins. As a regulator of β-catenin phosphorylation, LAR may therefore influence not only AMPAR delivery [109], but also synaptic adhesion. Presynaptically, PTP–cadherin interactions also regulate synaptic vesicle clustering. Activation of the Fer PTK by cadherin/p120catenin binding can recruit SHP2, which dephosphorylates β-catenin, allowing its entry into cadherin complexes and then recruitment of synaptic vesicles [113] (Fig. 4). Several recent studies have further implicated LAR RPTPs in synapse formation through cell-to-cell interactions. Woo identified a trans-synaptic interaction between LAR and netrin-G ligand-3 (NGL-3), an adhesion molecule that binds PSD95, inducing presynaptic differentiation [114]. Furthermore, PTPσ interacts with NGL-3 to promote synapse formation in a bidirectional manner, whereas the PTPδ–NGL-3 interaction triggers only presynaptic events [115] (Fig. 4). Recent work also shows that PTPδ controls synapse formation by trans-synaptic interactions with interleukin-1 receptor accessory protein-like 1 (IL1RAPL1) in mouse cortical neurons [116, 117]. IL1RAPL1 mutations are associated with autism and nonsyndromic X-linked mental retardation, suggesting that the function of PTPδ might also be disrupted here. Takahashi and coworkers have demonstrated, in hippocampal neurons, that the postsynaptic neurotrophic tyrosine kinase receptor type 3 (TrkC) binds the ectodomain of presynaptic PTPσ *in trans*, leading to the induction of presynaptic excitatory differentiation and postsynaptic clustering of synaptic components [118]. Like DLAR in flies, axonal PTPσ has other *trans* ligands, such as HSPGs and chondroitin sulfate proteoglycans (CSPGs) [106, 108] and these might potentially compete with TrkC binding. Synaptic HSPGs include syndecans, which regulate synaptic function [119]. PTPσ and LAR also interact with Trk proteins *in cis* [120, 121] and therefore complex, competitive interactions are likely. Another RPTP, PTPζ, is itself a CSPG. PTPζ associates with PSD95 and controls Rho-associated protein kinase and p190 Rho GTPase activating protein (RhoGAP). Gene-deficient mice have enhanced long-term potentiation (LTP) and deficits in learning, implicating PTPζ in synaptic plasticity [122].

Long-term depression

Long-term depression (LTD) at synapses is regulated in part by AMPAR phosphorylation and by active depletion of AMPAR numbers from synaptic membranes [123]. GluR2 subunits in adult hippocampi are subject to activity-dependent tyrosine phosphorylation during metabotropic GluR5 (mGluR5)-driven LTD [124]. GluR2 dephosphorylation causes AMPAR endocytosis [124, 125], and blockade of mGluR5-LTD by PTP inhibitors can in turn be prevented by Src kinase inhibitors [124, 126]. The major AMPAR PTP is cytoplasmic STEP. STEP loss-of-function ablates AMPAR internalization and GluR2 dephosphorylation [127]. AMPAR levels are also in part controlled in synapses by NMDARs. NMDARs containing NRB2 are tyrosine phosphorylated at a C-terminal YEKL motif by

Src kinases during synaptic activity, increasing the levels and activity of NMDAR in synapses [128]. STEP is a key negative regulator of NR2B, inducing dephosphorylation of YEKL and internalization of NMDAR. NMDAR activity also activates STEP [129], and STEP may then inactivate Fyn and dephosphorylate NRB2. In potentially the same complex, RPTPα binds PSD95 alongside Src kinases and can activate them, inducing NMDAR phosphorylation [130]. Interestingly, PTPα-deficient mice have NMDAR and LTP defects, arising from reduced activity in Src and also Pyk2, an NMDAR regulator [131]. Finally, LTD is also induced by mAChR receptor activation. This may follow association of AMPAR and the LAR/liprin-α complex, then dephosphorylation and internalization of AMPAR [110] (Fig. 4).

PTPs therefore not only regulate synapse formation, but also synaptic activity and are likely to have a broad influence over peripheral nervous system and CNS function. PTPs may well also be associated with neurological pathologies in addition to those in Table 1. To illustrate this, some novel findings implicating PTPs in CNS diseases are discussed below.

PTPs in CNS diseases

A high proportion of PTPs are expressed by neural cells, in neurons and in glia but also by inflammatory cells, microglia and T cells that can infiltrate the brain. As a consequence, PTPs have been implicated in various types of CNS disease, including neurodegenerative diseases, neuropsychiatric disorders and cancer. We will review PTPs in CNS disease, excluding gliomas because this has recently been dealt with [8].

RPTPZ and CD45 in inflammatory demyelinating diseases

The hallmark of inflammatory demyelinating diseases such as multiple sclerosis (MS) is the progressive destruction of oligodendrocyte (OLG)-produced myelin sheaths, resulting in physical and cognitive dysfunction. After a demyelinating insult, remyelination of damaged axons is thought to proceed in a similar manner as in development, but in the context of inflammation: the inflammation must be controlled and remyelination promoted. Inflammation causes nerve damage and consequently leads to T-cell infiltration into the CNS and the release of cytotoxic cytokines. Remyelination is carried by oligodendrocyte precursor cells (OPCs) that must differentiate into myelinating OLGs. Two PTPs have been implicated in these phenomena: RPTPZ and CD45.

As mentioned previously, subjecting PTPRZ knockout mice to experimental autoimmune encephalomyelitis (EAE), a well-established MS model, revealed a defect in remyelinating capacity *in vitro* and in the process of remyelination *in vivo*, which manifested as sustained paralysis associated with a loss of mature OLGs in the spinal cord. Examination of human MS lesions also showed increased expression of PTPRZ in remyelinating OLGs, supporting a role for PTPRZ in MS progression [132]. Although genetic linkage analysis for MS patients does not point to the PTPRZ locus (Table 1), RPTPZ has recently been shown to interact with the cell-adhesive neural recognition molecule contactin-1 (CNTN-1) [133], a biomarker for the progression of MS in adult cerebral spinal fluid [134]. Similarly, elevated levels of anti-RPTPZ Igs have been found in serum from patients with chronic inflammatory demyelinating polyneuropathy, an immune-mediated peripheral nervous system demyelinating disease [135]. Bidirectional signaling between CNTN-1 on neuronal axons and RPTPZ on the surface of OLGs may therefore guide the development and repair of myelin sheaths, making this interaction an attractive target for MS therapy [133].

The only PTP gene that has been genetically linked to MS susceptibility (Table 1) is *PTPRC*, which encodes CD45 [136]. While much work has been carried out studying CD45 in hematopoietic cell lineages, recent work has revealed expression of CD45 in neural stem cells and glial cells [137]. OPCs from CD45-deficient mice are defective in the ability to differentiate into mature, myelinating OLG, as a result of aberrant regulation of the Fyn kinase signaling pathway. Like the PTPRZ knockout mice, ultrastructural analysis of the myelin from CD45 knockout mice also exhibited a pattern of dysmyelination that persisted into adulthood [137].

Given that inflammatory demyelinating diseases involve the immune system, both RPTPZ and CD45 could play multiple roles in knockout mouse models of these diseases. CD45 is highly expressed in hematopoietic cells and microglia, and recent reports suggest a role for RPTPZ in the development of B cells [138] and other cells of the hematopoietic lineage [139]. The current mouse models for CD45 and PTPRZ do not differentiate roles for the different isoforms or cell types expressing these PTPs. Owing to the critical role of CD45 in T-cell differentiation, CD45 knockout mice are completely resistant to EAE challenge, yet mice expressing a point mutation in CD45 that leads to aberrant signaling display lupus-like symptoms and enhanced sensitivity to EAE challenge [140]. Therefore, better models, such as inducible systems or specific isoform knockin mice must be developed to address the

specific roles of RPTPZ and CD45 in inflammatory cells versus glial cells and neural stem cells upon exposure to the EAE model of MS.

Although not yet implicated in myelin disease, three PTPs have recently been shown to play a role in OLG development or regeneration: SHP2 promotes OLG maturation [141], DUSP6 modulates OLG death [142], and paired Ig-like receptor-B/SHP1 neuronal signaling cascades enhance axon regeneration [143]. Further studies are necessary to elucidate their roles in OLG regeneration in models of demyelinating diseases.

RPTPs in noninflammatory neurodegenerative diseases

Parkinson's disease is characterized by the death of dopaminergic neurons in the *substantia nigra pars compacta*, with early symptoms of involuntary movement, or dyskinesias, followed later by cognitive impairment. The cytokine pleiotrophin, an important factor in both CNS and peripheral nervous system development, has been found to be up-regulated in affected neural tissues from humans and in animal models of Parkinson's disease [144, 145]. One of the three receptors for pleiotrophin is RPTPZ, which is inactivated upon pleiotrophin-induced dimerization. To restore the signaling balance and thus survival of dopaminergic neurons, agonists of RPTPZ have been postulated as an attractive therapeutic target for Parkinson's disease [146].

Alzheimer's disease presents with progressive cognitive deterioration characterized by a loss of matter in the cerebral cortex and hippocampus, in addition to the hallmark beta-amyloid plaques and neurofibrillary tangles. The PTP STEP has emerged as a possible mediator of beta-amyloid-induced disruption in synaptic transmission. *PTPN5* encodes four STEP isoforms through alternative splicing, with the cytoplasmic STEP46 and membrane-bound STEP61 having phosphatase activity. STEP levels and activity have been found to be up-regulated in two mouse models of Alzheimer's disease (Tg-2576 and J20), and also in the prefrontal cortex of human patients. As STEP modulates AMPAR and NMDAR internalization (discussed earlier) this has direct bearing for LTP and LTD processes. A recent study showed that genetically decreasing the levels of STEP led to increased phosphorylation of STEP targets and restored cognitive functions in the Tg-2576-Alzheimer's disease model, a promising finding to target for Alzheimer's disease treatment. For additional roles of STEP in CNS disorders, including the cognitive dysfunction observed in schizophrenia, we refer to two excellent recent reviews [147, 148].

RPTPs in neuropsychiatric disorders

Depression is probably caused by both structural and functional defects in the brain. Major depressive disorder is unipolar depression in the absence of schizophrenia, bipolar or schizoaffective bipolar disorders; however, the different disease manifestations may share defects in some underlying pathways [149, 150]. *PTPRG* is structurally similar to *PTPRZ*, but unlike *PTPRZ*, *PTPRG* is predominantly expressed in neurons [151] and RPTPG binds to CNTN-3- to CNTN-6 [152, 153], whereas RPTPZ only binds to CNTN-1, indicating nonoverlapping functions. In a genome-wide screen in patients with bipolar disorder, PTPRG single nucleotide polymorphisms (SNPs) were significantly correlated with the schizoaffective type of bipolar disorder [154], and in another screen PTPRG SNPs were correlated with recurrent early-onset major depressive disorder, indicating that similar pathways may be affected in the two diseases [150].

Additional studies implicate the involvement of other PTPs in depression. In postmortem studies examining the prefrontal cortex of patients with major depressive disorder, protein levels of the well-studied tumor suppressor PTEN were increased and the enzymatic activity of its downstream effectors phosphoinositide 3-kinase and Akt/PKB were decreased, suggesting PTEN as an underlying cause of major depressive disorder [155]. PTPRR isoforms are highly expressed in the cerebellum, hippocampus and olfactory bulb and can interact with MAP kinases [21]. In postmortem studies, increased expression of PTPRR was found in the orbitofrontal cortex of suicide victims [156] and *PTPRR* SNPs may be associated with major depressive disorder in Caucasians [157] or in female subjects [149]; conformational studies with increased statistical power are needed to confirm this.

Additional CNS pathologies

Alcohol use disorders are genetically complex diseases, and neural signaling pathways, including those involved in regulating NMDARs, are implicated in the disease etiology. In a genome-wide association screen of patients with a high level of response to alcohol, nine RPTP genes were identified as important: *PTPRG*, *PTPRZ1*, *PTPRD*, *PTPRE*, *PTPRB*, *PTPRR*, *PTPRN*, *PTPRN2* and *PTPRT*, as well as the RPTPG-binding partner CNTN-4 [158]. STEP has also been associated with alcohol-induced memory loss [148] but the precise role of these individual PTPs in alcohol use disorders remains to be elucidated. Genetic work has linked CNTN-4 to both autism spectrum dis-

order [159] and 3p deletion syndrome [160], leading to the possibility that missense mutations in CNTN-4 affect the binding avidity of RPTPG. Given the association between RPTPs and the contactin family of cell-adhesion molecules, it is possible that many contactin-associated CNS diseases could be caused by aberrant interactions with RPTPs. A discussion of the full spectrum of CNTN-associated diseases is, however, beyond the scope of this review.

Conclusion

The work on cell and animal models and on hereditary disease states that we have discussed here, together with the many examples from acquired diseases, including cancer, that have recently been reviewed elsewhere [4–12], explain the increasing awareness of the importance of the regulatory potential of PTPs in health and disease. As an example we highlighted the role that several discrete PTPs play in osteoclast functioning. Such knowledge on individual PTPs is required in order to identify good targets for inhibitors that may be used for therapeutic gain and, no less importantly, to know which PTPs not to target.

Vertebrate PTPs exhibit strong and widespread expression during development of the nervous system. Previous research has revealed highly relevant roles for PTPs in axon growth and guidance systems. The spotlight has now swung onto a plethora of new roles for these enzymes in synaptogenesis and adult synaptic plasticity. This generates a gratifying complementarity with their axonal roles during development, once again highlighting how nature can efficiently redeploy proteins throughout ontogeny.

Maintaining proper CNS functioning throughout life represents an important health issue in current-day society. Broadly, two major defects underlie many of the CNS diseases: myelination defects and synaptic transmission impairments. Thus, a delicate interplay of glial cells and neurons is critical for maintaining a healthy CNS. Despite the currently limited genetic evidence that directly links PTP genes to specific CNS diseases, many of the neuronal-specific PTP mouse models displayed subtle phenotypes, over time or following proper challenges, that recapitulate progressive degenerative disease states. It is important to realize that human diseases manifest on a 'random bred' genetic background and in a highly variable environmental context, strongly opposing the uniform, well-controlled situation of animal models. It remains a challenge to unravel the minor alterations in CNS development and function that, exacerbated by genetic and environmental cues, can have such major consequences on human well-being.

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