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Invited Review

Protozoan protein tyrosine phosphatases

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Abstract

The aim of this review is to provide a synthesis of the published experimental data on protein tyrosine phosphatases from parasitic protozoa, in silico analysis based on the availability of completed genomes and to place available data for individual phosphatases from different unicellular parasites into the comparative and evolutionary context. We analysed the complement of protein tyrosine phosphatases (PTP) in several species of unicellular parasites that belong to *Apicomplexa* (*Plasmodium*; *Cryptosporidium*, *Babesia*, *Theileria*, and *Toxoplasma*), kinetoplastids (*Leishmania* and *Trypanosoma* spp.), as well as *Entamoeba histolytica*, *Giardia lamblia*, *Trichomonas vaginalis* and a microsporidium *Encephalitozoon cuniculi*. The analysis shows distinct distribution of the known families of tyrosine phosphatases in different species. Protozoan tyrosine phosphatases show considerable levels of divergence compared with their mammalian homologues, both in terms of sequence similarity between the catalytic domains and the structure of their flanking domains. This potentially makes them suitable targets for development of specific inhibitors with minimal effects on physiology of mammalian hosts.

Keywords: Signal transduction; Protein phosphorylation; Cdc25; Cdc14; PTEN; Myotubularin

1. Introduction

Protein phosphorylation is undoubtedly the most common and the best studied post-translational modification. It has been estimated that about 30% of all proteins can be regulated by phosphorylation (Hunter, 1995; Denu and Dixon, 1998). Although phosphorylation on Tyr comprises only a small fraction of all protein phosphorylation events (compared with Ser/Thr phosphorylation), it plays a disproportionately important role in such aspects of signalling as cell-cycle control or differentiation. Therefore, interfering with protein phosphorylation represents a potentially powerful pharmacological approach. Indeed, protein kinases have recently become one of the two most important groups of drug targets (Cohen, 2002). In particular, protein kinases of parasitic protozoa have been actively discussed as potential targets for antiparasitic drugs (Doerig et al., 2002; Canduri et al., 2007; Doerig and Meijer, 2007). Since the phosphorylation status of any protein is controlled by both kinases and phosphatases, the latter can be exploited as therapeutic targets as well (van Huijsduijnen et al., 2002; Barr and Knapp, 2006; Easty et al., 2006; Tautz et al., 2006).

Protein Tyr phosphatases (i.e. phosphatases able to dephosphorylate phosphotyrosyl residues) belong to three evolutionarily unrelated classes: protein tyrosine phosphatases (PTPs), Cdc25 and low molecular weight phosphatases (LMW-PTPs). They share a common motif (CX_5R) in their catalytic centres and common cysteine-based mechanism of catalysis, which probably originated by convergent evolution (Denu and Dixon, 1998; Alonso et al., 2004). In humans, the Cdc25 family contains three genes and LMW-PTPs are represented by a single gene, although several LMW-PTP isoforms originate by alternative splicing (Bottini et al., 2002; Raugei et al., 2002; Donzelli and Draetta, 2003; Karlsson-Rosenthal and Millar, 2006). In contrast, the PTP class is represented in humans by >100 genes, which are further subdivided into PTPs in the

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narrow sense (classical PTPs) and dual specificity phosphatases (DSPs) (Alonso et al., 2004). Classical PTPs are strictly Tyr-specific and are further subdivided into receptor type (with transmembrane domains) and non-receptor type (without transmembrane domains). DSPs may dephosphorylate different combinations of Tyr and Ser/Thr, as well as non-protein substrates. Human DSPs fall into several groups (Alonso et al., 2004): (i) mitogen-activated protein (MAP) kinase phosphatases (MKPs), which as the name implies dephosphorylate MAP kinases ERK, JNK and p38 and have a characteristic inactive rhodanese (sulphurtransferase) homology domain related to Cdc25; (ii) "atypical" DSPs, a group that is not well defined and combines DSPs that do not clearly belong to other groups; some of these phosphatases are closer to MKPs in terms of the primary structure of their catalytic domains, but do not have rhodanese-homology domains; (iii) slingshots, which specifically dephosphorylate an actin-binding protein cofilin (Pak et al., 2008); (iv) phosphatases of regenerating liver (PRLs), C-terminally prenylated phosphatases involved in regulation of cell proliferation, migratory and invasive properties (Stephens et al., 2005); (v) Cdc14s, which regulate centrosome cycle and cell division (Trautmann and McCollum, 2002; Stegmeier and Amon, 2004; Sullivan and Morgan, 2007). In addition, DSPs include two groups of lipid phosphatases, phosphatase and tensin homologues (PTENs) and myotubularins (Alonso et al., 2004). Several groups of tyrosine phosphatases contain catalytically inactive members or domains (sometimes termed "anti-phosphatases"), which may play a role in substrate binding or function as adaptors for their active counterparts (Hunter, 1998; Begley and Dixon, 2005). In addition to the three "cysteine"-based classes, other groups of unrelated enzymes such as Eya or acid phosphatases may also function as protein Tyr phosphatases (Rebay et al., 2005; Veeramani et al., 2005).

In recent years, genome sequences of several evolutionarily divergent parasitic protozoa have been completed, namely several species of *Apicomplexa* (Carlton et al., 2002; Gardner et al., 2002, 2005; Abrahamsen et al., 2004; Xu et al., 2004; Pain et al., 2005; Brayton et al., 2007) and *Kinetoplastida* (Berriman et al., 2005; El-Sayed et al., 2005; Ivens et al., 2005; Peacock et al., 2007), as well as *Entamoeba histolytica* (Loftus et al., 2005), *Giardia lamblia* (Morrison et al., 2007), *Trichomonas vaginalis* (Carlton et al., 2007) and a microsporidium *Encephalitozoon cuniculi* (Katinka et al., 2001). This opens extensive possibilities for exploration of various aspects of cell physiology of these organisms, which may eventually be exploited to develop new therapeutic approaches.

Several in silico studies have analysed the protein kinase complements ("kinomes") of parasitic protozoa (Ward et al., 2004; Parsons et al., 2005; Miranda-Saavedra et al., 2007). Another recent work has provided analysis of the protein phosphatase complements ("phosphatomes") of three kinetoplastid species (TriTryps: *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major*) (Brenchley et al., 2007).

The aim of this review is to provide a synthesis of the published experimental data on protein Tyr phosphatases from parasitic protozoa and in silico analysis based on the availability of completed genomes, and to place available data for individual phosphatases from different unicellular parasites in the comparative and evolutionary context.

2. PTPs

2.1. "Non-receptor" type PTPs

"Non-receptor" type PTPs are the only type present in protozoa, as they do not have PTPs with transmembrane domains (i.e. "receptor type").

Although kinetoplastids do not have tyrosine kinases (Parsons et al., 2005), their genomes encode three (*Leishmania*) or two (*Trypanosoma*) protein tyrosine phosphatases, which fall into three separate clades characterised by specific patterns of conservation of distinctive motifs (Brenchley et al., 2007; Fig. 1).

Trypanosoma brucei PTP (TbPTP1) exhibits enzymatic properties expected for classical PTPs, i.e. preferential dephosphorylation of phospho-Tyr-containing peptides and inhibition by known PTP inhibitors (Szoor et al., 2006). Similar to classical PTPs, TbPTP1 is reversibly inhibited under oxidising conditions. TbPTP1 is expressed throughout the *T. brucei* life cycle and is predominantly associated with the cytoskeleton. RNA interference (RNAi)-mediated TbPTP1 depletion or its inhibition by a PTP1-specific inhibitor BZ3, results in a spontaneous differentiation of bloodstream *T. brucei* into a proliferative procyclic form (Szoor et al., 2006). This role of TbPTP1 seems to be specific, since RNAi-mediated depletion of the second *T. brucei* PTP isoform, TbPTP2 (see Fig. 1), does not result in differentiation of bloodstream forms (Szoor et al., 2006). Thus, the physiological role of TbPTP1 appears to be in hindering differentiation of otherwise committed cells. The authors speculate that uptake by the tsetse fly results in TbPTP1 inhibition, possibly due to alkaline pH or oxidising conditions in the fly's digestive tract, and this triggers *T. brucei* differentiation (Szoor et al., 2006).

Leishmania major PTP (LmPTP1, note that it is not an orthologue of TbPTP1, as shown in Fig. 1) also shows enzymatic characteristics of a typical PTP (Nascimento et al., 2006). *Leishmania donovani* PTP1 (LdPTP1) is closely related to LmPTP1 (Fig. 1). Deletion of its gene only slightly decreases the proliferation rate of both promastigotes and amastigotes in cell cultures, but strongly impairs survival of amastigotes in mice, resulting in a 80–90% reduction in virulence (Nascimento et al., 2006). This phenotype can be complemented by re-introduction of a plasmid with LdPTP1 cDNA. In line with these findings are previous observations by the same group that expression of human PTP1B in *L. donovani* increases virulence (Nascimento et al., 2003). It has been speculated that the function of these phosphatases (LmPTP1 and TcPTP1) may be related to intracellular parasitism (Brenchley et al., 2007).