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Review

A *Drosophila*-centric view of protein tyrosine phosphatases

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ABSTRACT

Most of our knowledge on protein tyrosine phosphatases (PTPs) is derived from human pathologies and mouse knockout models. These models largely correlate well with human disease phenotypes, but can be ambiguous due to compensatory mechanisms introduced by paralogous genes. Here we present the analysis of the PTP complement of the fruit fly and the complementary view that PTP studies in *Drosophila* will accelerate our understanding of PTPs in physiological and pathological conditions. With only 44 PTP genes, *Drosophila* represents a streamlined version of the human complement. Our integrated analysis places the *Drosophila* PTPs into evolutionary and functional contexts, thereby providing a platform for the exploitation of the fly for PTP research and the transfer of knowledge onto other model systems.

1. Introduction

Reversible protein phosphorylation is one of the most widespread mechanisms for controlling cellular functions. Tyrosine phosphorylation, in particular, has evolved into a sophisticated regulatory system in metazoans to control many animal-specific processes, ranging from development to cellular shape and motility, transcriptional regulation and proliferation versus differentiation decisions [1]. Out of 498 genes encoding protein kinases in the human genome, 91 encode tyrosine kinases (TKs) [2]. The set of TKs is complemented with 109 protein tyrosine phosphatase (PTP) genes [3], although TKs and PTPs do not seem to have overlapping targets.

Given the central importance of tyrosine phosphorylation, it is no surprise that its abnormal regulation is responsible for many human diseases: diabetes, obesity, cancer, inflammatory diseases, and many others have been associated with PTP over-expression or deficiencies in human [4]. Historically research on TKs has advanced at a faster rate than that on PTPs; not only were TKs identified nearly a decade earlier than PTPs, but also the intrinsic difficulties of investigating the "disappearance" of a phosphate moiety as opposed to the appearance of a radioactive phosphate represented a major burden for the PTP field. Major advances have

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been made with the development of substrate trapping techniques where specific mutations of the PTP catalytic domains allow the purification, detection and identification of their physiological substrates. Although PTPs are generally expressed at low levels, they are enzymatically very active and as a result their ectopic expression in cellular systems easily leads to off-target effects, usually resulting in cell death [5]. Therefore, the use of model organisms is indispensable for the study of PTPs.

Today we know that in most cases phosphatases play a dominant role over protein kinases in shaping the spatio-temporal dynamics of protein phosphorylation networks, which are characterized by their amplitude and duration [6]. Thus it has been proposed that in many instances protein phosphatases make better drug targets than protein kinases. Most of our current knowledge on PTPs is derived from mutations identified in human pathologies as well as loss-of-function studies in the mouse (embryonic gene targeting models, and siRNA and shRNA knockdown experiments). To a large extent, the murine genetic deletions correlate well with human disease phenotypes and have been instrumental in understanding the central importance of PTPs in cellular signaling, whose effects can range from embryonic-lethal to relatively mild and nearly unnoticeable likely due to the presence of compensatory mechanisms by paralogous PTP genes [4,5]. Whereas the mouse has so far been the favoured organism for the genetic dissection of PTP-controlled pathways, here we argue that *Drosophila melanogaster* with its streamlined version of the human tyrosine phosphatome is a complementary and powerful system for the dissection of PTP functions in vivo.

The fruit fly occupies a paramount position among model organisms: *Drosophila* research yielded the first observations on a wide variety of fundamental biological principles that are conserved in humans despite several hundred million years of independent evolution [7–9]. These include the principles of embryonic patterning by Hox genes, which apply to all bilaterian animals [10], and the functional conservation of other key signaling pathways, including among others: (i) the Notch signaling pathway [11–22]; (ii) the Wnt set of signal transduction pathways, which function in various developmental pathways (body-axis patterning, cell migration, cell fate specification, cell proliferation; and (iii) the identification of the *Drosophila* receptor tyrosine kinase gene *Sevenless* (*sev*), whose target protein Son of sevenless (Sos) unveiled the relationship between receptor TK and Ras signaling in eye development [23]. These conserved signaling pathways are frequently dysregulated in cancers and type II diabetes [24–27]).

The main advantage of the fly over other model organisms is that once a suitable phenotype has been obtained, its genetic toolkit can be used to dissect the underlying disease pathways either by loss-of-function (by RNAi, transposon insertion, imprecise excision, or X-ray and chemical mutagenesis [28]) or gain-of function in a tissue-specific manner (e.g. using the GAL4/UAS system [29]. Thus, *Drosophila* is a particularly attractive system for establishing models of human disease and studying genetic interactions for a number of reasons [8]: (i) many well-studied biological pathways are conserved between human and *Drosophila*, including pattern formation, endocrine and intracellular signaling, and cell death [30]. Furthermore, in recent times the fruit fly has emerged as an extraordinarily powerful model for the study of human metabolism, where a homolog of leptin (a hormone that regulates energy intake and expenditure, including appetite, hunger, metabolism and behavior) called *unpaired 2* (*upd2*, a JAK-STAT pathway ligand) works similarly to human leptin [31]; (ii) over 75% of human disease-associated genes have a homolog in the fly [32], and as such fly models have been successfully established for many distinct diseases, including neurodegenerative disorders, cancer, cardiac, immunological and developmental disorders [33]; (iii) its short life cycle and abundant progeny facilitates genetic modeling, whereas its relatively short lifespan (~120 days) makes studies on ageing particularly feasible.

Here we describe the PTP complement of *D. melanogaster*, a model organism where studies on tyrosine phosphatases have previously shed light on key pan-metazoan functions. We draw similarities and differences between the tyrosine phosphatome of the fruit fly and those of human, mouse, worm and yeast, highlighting important conserved functions that underline the potential of the fruit fly as a key model organism for the genetic and biochemical study of tyrosine phosphatases in higher animals.

2. A revised annotation of the *Drosophila* tyrosine phosphatome

The PTP superfamily is divided into 4 distinct classes that differ both in their catalytic mechanisms and phosphatase catalytic domain sequences [34]. Class I are cysteine-based PTPs including the classical tyrosine-specific phosphatases (both receptor and non-receptor), and the dual-specificity phosphatases (DSP, or VH1-like). DSPs are the most promiscuous type of PTPs in terms of substrate specificity, with some members dephosphorylating mRNA 5′-triphosphate while other enzymes dephosphorylate lipids. Class II PTPs are a small but evolutionarily highly conserved group of PTPs with only one member in humans (ACP1); they are also found in bacteria (which display tyrosine phosphorylation [35]) and are structurally related to bacterial arsenate reductases. Class III PTPs, like the Class I and II, are also cysteine-based enzymes displaying specificity towards tyrosine and threonine residues. The human enzymes (CDC25A, CDC25B and CDC25C) control cell cycle progression by dephosphorylating cyclin-dependent kinases. Despite sharing a cysteine-based catalytic mechanism, Class I, II, and III PTPs are believed to have evolved independently. A fourth class of PTPs displays an aspartic acid-based catalytic mechanism with dependence on a cation, and is represented by the developmentally important EyA ('Eyes Absent') genes, of which only one member is found in the fruit fly compared to four genes in mouse and human.

We recently developed a highly sensitive and specific method for the automatic classification of proteins into the various PTP classes and families ('Y-Phosphatomer'). Y-Phosphatomer relies on a specific collection of protein domain models drawn from InterPro member databases [3]. Upon evaluation, Y-Phosphatomer reported perfect coverage and classification rates. Then, as proof of principle we reannotated the human tyrosine phosphatome and showed that the human genome harbors 109 PTP genes instead of 105 genes as originally reported in a hallmark paper 10 years ago [34]. We subsequently used Y-Phosphatomer to annotate the PTP complements of 65 eukaryotic genomes (including *Drosophila*), which are available through the PTP-central database (http://www.PTP-central.org/) [3]. The *D. melanogaster* genome contains 44 PTP genes (Fig. 1A), with RNA-seq data from FlyBase [36] supporting both the robust expression and intron–exon structures of all PTPs genes. The curated tyrosine phosphatome of the fly presented here extends the previous catalogue of 16 (classical) tyrosine-specific PTPs [37] by 21 dual-specificity phosphatases (DSPs), 4 Class II phosphatases (LMWPs), 2 Class III phosphatases (CDC25s) and 1 eyes-absent (EyA) homolog (Table 1).

Previous efforts at characterizing the PTP complement of *D. melanogaster* include those by Andersen et al. [37] and Morrison et al. [38]. While the former study exclusively concentrates on Class I tyrosine-specific PTPs (and correctly identifies all 16 genes in *Drosophila*), the latter is much broader in scope and attempts to identify all protein kinases and phosphatases in the fly genome. To do so, Morrison and colleagues mined the fly genome using