

The characterization of novel protein phosphatases in *Drosophila* species

by Csaba Ádám, M.Sc.

Department of Medical Chemistry, Medical and Health Science Center University of Debrecen

Supervisor: Viktor Dombrádi, Ph.D, D.Sc.

DOCTORAL SCHOOL OF MOLECULAR MEDICINE

Protein phosphorylation and dephosphorylation are common gears of regulation in all eukaryotic organisms. The group of the so-called phosphoprotein phosphatases (PPP) removes the phosphate from the Ser and Thr residues of proteins. The PPP family comprises classical members that were identified by biochemical assays and novel enzymes that were discovered by molecular biology or genetic approaches.

In the genome of *Drosophila melanogaster* we have identified 19 phosphoprotein phosphatase (PPP) catalytic subunit coding genes. Seven of the novel members of the gene family turned out to be *Drosophila*-specific. *CG11597* is a recently evolved gene that is expressed during all stages of morphogenesis in *D. melanogaster*. In contrast, transcription of the *PpD5*, *PpD6*, *Pp1-Y1*, and *Pp1-Y2* genes is restricted to the pupa and imago developmental stages and to the testes of the males, just as that of the previously characterized *PpY-55A* and *PpN58A*. The mRNA of *PpD5*, *Pp1-Y1*, and *PpY-55A* were detected in the developing cysts by *in situ* hybridization, in contrast with the *PpD6* transcript that was found in the distal ends of elongating spermatids. The localization suggests that *PpD6* is one of the few post-meiotically transcribed genes in *D. melanogaster*.

Based on the genome sequences of 12 *Drosophila* species we traced the evolution of the PPP catalytic subunits. We noted a substantial expansion of the gene family. We concluded that the 18-22 PPP genes of *Drosophilidae* were generated from a core set of 8 indispensable phosphatases that are present in most of the insects. Retropositions followed by local gene duplications extended the basic phosphatase repertoire, and sporadic gene losses contributed to the species specific variations in the PPP complement. During the course of these studies we identified 5 up till now uncharacterized phosphatase retrogenes: *PpY+*, *PpD5+*, *PpD6+*, *Pp4+*, and *Pp6+*, which are found only in some ancient *Drosophila* species. We demonstrated that all of these new PPP genes exhibit a distinct male specific expression. Our data support the “out of testis” hypothesis suggesting that the new functional retrogenes are preferentially transcribed in the male gonads. We have also proved that the sequence of novel, male-specific phosphatases changed more rapidly than that of the classical phosphatases, thus our results support the “faster male” hypothesis. In addition to the changes in gene numbers, the intron-exon structure and the chromosomal localization of several PPP retrogenes was also altered during evolution. The G-C content of the coding regions decreased when a gene moved into the heterochromatic region of the Y chromosome. In conclusion, the PPP enzyme family exemplifies the various types of dynamic genome rearrangements that accompany the molecular evolution of the novel retrogenes in *Drosophilidae*.

Keywords: *Drosophila*, Ser/Thr protein phosphatase, evolution

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