THESIS FOR THE DEGREE OF DOCTOR OF PHYLOSOPHY (Ph.D.)

Functional analysis of novel protein phosphatases in *Drosophila melanogaster*

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1. INTRUDUCTION

Protein phosphorylation-dephosphorylation

Phosphorylation is a covalent protein modification evolved in the early stages of the evolution. Many examples have recently suggested the significance of phosphorylation in prokaryotes. Phosphorylation became a successful process in the evolution because protein kinases can be found in all eukaryotic cells and their structure originates from a common anchestor. Binding of the negatively charged phosphate group to the Ser, Thr or Tyr amino acids will cause a conformational change of the protein structures. The phosphate group can stabilize the structure of the protein, increase its nutritional value, and play a regulatory role but sometimes it has no effect or the physiological role is not known. Protein phosphorylation could only become an effective regulatory process in a reversible way. Dephosphorylation is performed by protein phosphatase enzymes that can hydrolyze phosphate group from the proteins. Protein kinases and phosphatases are equivalent components of the regulatory systems. Based on the substrate specificity, the structure of the catalytic subunit of the protein phosphatases and the reaction mechanism we can classify them into the phosphoprotein phosphatase (PPP), metal ion dependent protein phosphatase (PPM) and the RNA polymerase II C-terminal domain specific protein phosphatase (FCP) enzyme family. Protein tyrosine phosphatases (PTP) can dephosphorylate the Tyr sidechain of the proteins using active cysteine for their catalytic mechanism and producing cysteine-phosphate intermediate. The reaction mechanism of the dual specific protein phosphatases is identical with that of the PTP enzymes, but they can dephosphorylate Ser/Thr or Tyr side chains.

Phosphoprotein phosphatase (PPP) enzyme family

The Ser/Thr specific phosphoprotein phosphatases (PPP) constitute a highly conserved enzyme family. Their representatives are present in ancient archea, but do not play essential functions in the bacteria. The classical members of the family (PP1, PP2A, and calcineurin/PP2B) were detected by traditional biochemical methods. PP1 and PP2A play essential role in the eukaryotic cells. Although inhibition of PP2B is not lethal, still it has an important regulatory role. Additional, “novel” phosphatases were discovered by molecular cloning and genetic approaches. According to the structures of their catalytic domains, these novel enzymes represent transitions between the classical PP1 and PP2A. Although their activity can hardly be measured with the commonly used substrates, they have distinct and important physiological roles. Some of them exhibit broad phylogenetic distribution (PPX/PP4, PPT/PP5, Sit4/PPV/PP6 and rdgC/PP7), while others are restricted to a given kingdom or to a given species. In *Drosophila melanogaster* there are two species-specific enzymes, protein phosphatase Y (PPY) and N (PPN). Our studies shall concentrate on these two protein phosphatases.

Drosophila PPP enzyme family

Before the completion of Drosophila genome completed, most of the PPP members had been identified by library screening, PCR and classical genetic methods on the bases of structural similarities. Sequencing data allowed the identification of new members of the protein phosphatase family. The Drosophila genome contains 19 genes encoding catalytic subunits of PPP phosphatases; whose products are four PP1 isoforms, PP2A (mts) and three PP2B (CanA) isoforms belonging to the classical protein phosphatases. The novel types are PP4, PP5 (PpD3), PP6 (PPV), PP7 (rdgC), PPN, PPY, PpD5 PpD6 and the gene product of CG11597, as well as two protein phosphatases located at the Y chromosome. The function of the novel enzymes are less characterized.

Drosophila specific protein phosphatases

Protein phosphatase Y (PPY) and N (PPN) were cloned from a *Drosophila melanogaster* cDNA library and were shown to be similar to PP1 according to the predicted primary structure of the encoded proteins. The corresponding genes termed PpY-55A were localized at position 55A and PpN-58A was localized at position 58A on the cytogenetic map. Analysis of the genomic sequence showed that neither PPY nor

PPN genes contain introns and are present only in one copy in the Drosophila genome. The PPY and PPN mRNA transcripts were detected in the male flies after the late larval developmental stage. Both phosphatase genes are specifically expressed in the testis of third instar larvae, pupae and imago. The PPY protein was reported to accumulate in the nuclei of cyst cells by immunohistochemical method. Based on its expression pattern it was suggested that PPY may be involved in spermatogenesis. The enzyme activity of the recombinant protein can be measured with external substrates. As expected, the enzyme activity was inhibited by okadaic acid and was hardly affected by inhibitor-2/PP1R2. PPN specific antibody has been not developed, therefore the expression pattern of the protein is unknown. Since no PPY and PPN mutants have been isolated, the exact function of these novel phosphatases remains an open question.

Regulation of protein phosphatases

The data of recently finished genome projects revealed a larger number of genes encode protein kinases than protein phosphatases. On the basis of the genome sequencing data the ratio between protein kinases and phosphatases is ~10:1 in the human and ~6:1 in the Drosophila. How can the protein phosphatases act as equivalent regulators with protein kinases, even though they have much smaller number? The answer can be given by the large number of the protein phosphatase regulators that increase the specificity of the catalytic subunits. In the case of PP1 has been identified more then fifty regulators that consist conserved recognition site for the phosphatase. PP2A exists as a holoenzyme composed of catalytic (C) subunit, a scaffolding A subunit, and several variable B subunits (B', B'', B''') or other the proteins. The PP2B holoenzyme consists of a catalytic (A) and a regulatory (B) subunit. Ca-calmodulin stimulates the catalytic activity of this dimeric core. The regulators of protein phosphatase catalytic subunits change the enzyme activity, substrate specificity and direct the enzyme activity to different cell compartments.

Regulators of the novel protein phosphatases

The regulatory proteins of the novel PPP members have been less characterized. PPP4 has three distinct regulatory subunits: α4, $PP4_{RI}$ and PP4R2. In addition, PP4 interacts with the transcription factors c-Rel, NFκB p50, and RelA, with the Survival of Motor Neurons complex, and with insulin receptor substrate 4. PP5 binds to Hsp90-glucocorticoid receptor heterocomplexes, to cryptochrome 2, to the CDC27 subunit of the anaphase-promoting complex, to the apoptosis signal-regulating kinase 1, to the Gα12 and Gα13 subunits of heterotrimeric G proteins, to copines I, II, and IV, to the checkpoint kinase ATM, as well as to the catalytic subunit of DNA dependent protein kinase. A series of SAP proteins (SAP155, SAP185, SAP190) associate with SIT4/PP6. The inhibition of Ppz1, a fungus specific novel protein phosphatase by Hal3 and its homologue Vhs3 was reported. The novel protein phosphatases play an important role in many biochemical processes. The identification of the novel phosphatase regulators can be useful in determining the exact function of the holoenzyme. In the present communication we characterize the Drosophila PPY and PPN interacting proteins.

2. AIMS

The protein phosphatase Y and N are expressed only in the testis of the adult flies and their function is still unknown. We were interested in the regulatory pathways opened up by the appearance of the novel PPY and PPN besides the classical protein phosphatases. Our immediate goals were the following.

1. To identify proteins that interact with PPY and PPN by the yeast two-hybrid method.

2. To confirm the putative interactions.

3. To select a PPY interacting protein for further analysis and check the specificity of the interaction.

4. Biochemical characterization of the interactor protein.

5. To develop specific antibody against the protein.

6. To determine the expression pattern of the PPY interactor and to localize it.

3. METHODS

Vector constructions

Since the PPY and PPN genes contain no introns in their coding regions, a Drosophila genomic DNA preparation was suitable for PCR amplification of its entire ORFs. The coding region of the PPYR1 was PCR amplified from the AT19571-pOTB7 plasmid. The cDNA of PPY and PPN were ligated into the yeast two-hybrid pGBKT7 bait vector containing GAL4 DNA binding domain and the *Bgl*II - *Bcl*I fragment of the AT19571-pOTB7 plasmid was ligated into the pACT prey vector containing the GAL4 trans-activator domain. In order to express the PPY, PPN and PPYR1 proteins their coding cDNA PCR fragments were inserted into the pET28a vector. The coding regions of PPY or PPN were cloned into the multiple cloning site of a modified pcDEF3-HA vector and the ORF of PPYR1 was inserted into a modified pcDEF3-Myc vector that allowed the expression of a fusion proteins in COS-7 mammalian tissue culture cells.

Yeast two-hybrid experiments

A 3rd instar larval Drosophila cDNA library was cotransformed with PPY- or PPN-pGBKT7 vector into Y190 yeast cells. The cells were tested in the appropriate synthetic complete media in the presence of 3-amido-1,2,4-triazole for His autotrophy and for β-galactosidase activity. Double positive yeast cells containing the cDNA of putative PPY and PPN interacting proteins were selected and their plasmid content was isolated and amplified in XL-10 Gold *E. coli* cells. We obtained 21 positive colonies realeted to PPY and 5 positive colonies realeted to PPN and we identified the inserted cDNAs by sequencing. One of the PPY positive clones derived from the gene CG15031 (termed PPYR1) was analyzed in detail.
The PPY-pGBKT7 or PPN-pGBKT7 construct as well as the pAS2-PP1α87B, pAS2-PP1β9C, pAS2-PP1α13C, pAS2-PP1α96A Drosophila protein phosphatase 1 catalytic subunit isoforms in the bait plasmid constructs were co-transformed into Y190 yeast strain with the pACT- PPYR1 prey in a pair-wise fashion and tested for two-hybrid interactions.

Expression of recombinant proteins

Recombinant His-PPY, His-PPN and His-PPYR1 proteins were expressed in *E. coli* BLR strain according to the instruction of the pET protein expression system. COS-7 mammalian tissue culture cells were transformed with the pcDEF3-HA-PPY, pcDEF3-HA-PPN or pcDEF3-Myc-PPYR1constructs using FuGENE 6 Transfection Reagent. Cells were maintained in DMEM media in the presence of glutamine, FBS and gentamycine.

Nucleic acid analyzing methods

Genomic DNA was isolated from adult flies and total RNA was purified from Drosophila testis, ovary and embryos. The *Hind* III, *Xho* I, *Eco* RI restriction fragments of the genomic DNA and the isolated total RNA were transferred to Hybond-N^+ nylon membrane and was hybridized with radiolabeled full length cDNA of PPYR1 for Southern and Northern blot. For the RNA in situ hybridizaton AT19571-pOTB7 plasmid was linearized by *Eco*RI digestion and used as a riboprobe. Antisense digoxigenin-labelled RNA probes were synthesized using the T7 promoter.

Protein analyzing methods

The concentration of the affinity purified and dialyzed proteins were determined by the Bradford method. The size of the proteins were defined by SDS-PAGE using standard molecular-weight protein mixture. Recombinant His-PPYR1 was analyzed by matrix assisted laser desorption ionization time of flight mass spectrometry. The CD spectra of the PPYR1 was recorded in the absence or presence of total *Drosophila* RNA. We analyzed the kinetic parameters of the PPY-PPYR1 interaction by Biacore methods. We also comfirmed the PPY-PPYR1 interaction by "pull-down" method. COS-7 cells were transfected with pcDEF3-Myc-PPYR1 vector. Either His-PPY or His-PPN purified recombinant protein was mixed with the supernatant of the Myc-PPYR1 producing cells. Ni-NTA agarose was added to the mixture and the resin was collected by centrifugation. The samples were analyzed by Western blot using anti-My antibody. The protease sensitivity of PPYR1 was analyzed by trypsin, proteinase K and 20S proteasome treatment. The phosphorylation of the PPYR1 was performed by cAMP-dependent protein kinase in the presence of [γ^{32}P]-ATP. The incorporated radioactivity was determined by Cherenkov radiation and was detected by autoradiography. The activity of renatured recombinant PPY was assayed with myelin basic protein that was phosphorylated by the catalytic subunit of PKA and [γ^{32}P]-ATP.

Immunological methods

In order to confirm the PPY-PPYR1 interaction we immunoprecipitated the lysate of the COS-7 cells that expressed the HA-PPY and Myc-PPYR1 proteins by anti-HA antibody. The PPYR1 protein was detected by Western blot using anti-Myc antibody. The PPYR1 specific polyclonal antibody was developed in rabbit. The affinity purified immunoglobulines were used for Western blot and immunohistochemistry studies. The proteins were separated by SDS-PAGE and transfered to the nylon membrane. The recombinant proteins were detected by anti-Myc rabbit polyclonal, anti-HA mouse monoclonal and anti-penta-His mouse monoclonal antibodies. The native PPYR1 was identified by anti-PPYR1 rabbit polyclonal antibody. The affinity purified anti-PPY and anti-PPYR1 were used for the immunohistochemical analysis of Drosophila embryo, testis and ovary. The localization of PPY and PPYR1 were determined by a confocal microscope.

4. RESULTS

Identification of PPY interacting proteins

In a yeast two-hybrid screen when the full length coding regions of PPY and PPN were used as bait we identified five PPY and one PPN binding proteins. The protein phosphatase Y bound to CG15031 gene product homologue of the *Vasa* intronic gene, putative RNA binding protein, to CSN5 component of the signalosome (CG14884), to homospermidine synthetase enzyme (CG4362), to an aminoacid transporter (CG7255) together with a PP1 binding protein (CG1553). Interacting with PPN we could detect only the gene product of the CG6167 that showed 65% homology with human PKCα binding protein (PICK1).

Confirmation the specificity of the PPY-CG15031 interaction

We selected the CG15031 gene product interacting with PPY for further investigation. We verified the data of the Drosophila Flybase database that one CG15031 gene can be found in the genome by Southern blot. The gene located at 13B3 region of the Drosophila X chromosome. The CG15031 cDNA had been previously isolated from Drosophila testis. Since PPY was detected in this organ, we deemed the interactions could physiologically be relevant. The specificity of PPY -PPYR1 interaction was tested by the yeast two-hybrid system. We found that neither the Drosophila PP1 catalytic subunit four isoforms nor PPN interacted with CG15031; under the same conditions PPY gave a clear positive signal. Based on these observations CG15031 was designated as a PPY interacting regulator protein 1 (PPYR1). The results of the two-hybrid experiments were confirmed by two independent methods. First, HA-tagged PPY and Myc-tagged PPYR1 were co-transformed into COS-7 cells. After co-expression an *in vivo* interaction between the two proteins was demonstrated by co-immunoprecipitation with an anti-HA antibody. Thus, Myc-PPYR1 bound across HA-PPY protein to the immunocomplex. Second, in a pull down experiment His-tagged bacterially expressed PPY was added to the extracts of COS-7 cells producing Myc-PPYR1. The His-tag of the fusion protein was used for binding PPY to Ni-agarose and the Myc-tag of the associated PPYR1 was detected in the pellet. The selectivity of the complex formation was confirmed by the lack of specific immunoreaction when His-PPN was used instead of His-PPY. The PPY-PPYR1 interaction was confirmed by three independent methods.

The physical interaction between His-PPY and His-PPYR1 was tested in a Biacore apparatus. The calculated dissociation constant (K_d) for the protein complex was $(6.2 \pm 1.6) \times 10^{-8}$ M (n=3). The much larger K_d value of $(4.2 \pm 0.6) \times 10^{-4}$ M (n=3) determined for the PPN-PPYR1 complex under similar conditions underlines the specificity of the interaction.

Biochemical characterization of PPYR1

Based on the gene and cDNA sequences of PPYR1, a protein of 309 amino acids has been predicted as the only gene product. No mutation affecting the gene has been described and the function of the gene product is unknown. In order to characterize the biochemical properties of the PPYR1 we expressed the protein in *E. coli*. The amino acid sequence of the purified protein was checked by mass spectroscopy. 28 peptides covering 77% of the expected sequence had the predicted mass values. The primary structure of PPYR1 was the same as coded by the cDNA consequently it was suitable for *in vitro* biochemical characterization of PPYR1. From the deduced amino acid sequence of PPYR1 a molecular mass of 33 kDa was predicted. Contrary to expectations, the experimentally determined molecular mass of the protein generated either in mammalian or in bacterial expression systems was 47 kDa. The larger than expected apparent molecular mass is characteristic of intrinsically unstructured proteins. We confirmed the disordered structure of PPYR1 by independent methods. The recombinant PPYR1 has a CD spectrum with a negative maximum at 200 nm which underscores that the protein lacks significant repetitive structure and is dominated by the coil conformation. It is highly sensitive to proteases as compared to BSA this is another characteristic feature of unstructured proteins. PPYR1 was effectively destroyed by purified 20S proteasome even in the absence of ubiquitination. The latter reaction was blocked by epoxomicin, a specific proteasome inhibitor. It is to be noted that disordered proteins, such as alpha-

synuclein and $p21^{Cip1}$ are effectively degraded by the 20S proteasome without prior ubiquitination. Many unstructured proteins are heat resistant. The heat stability of PPYR1 was demonstrated by boiling. Thus, the unstructured nature of the protein was confirmed by several lines of experimental evidence.

RNA binding capacity of PPYR1

Based on sequence comparisons, PPYR1 belongs to the family of hyaluronan/RNA binding proteins. The predicted RNA binding domain in the primary structure of PPYR1 was manifested. Addition of His-PPYR1 to purified *Drosophila* total RNA preparation resulted in a CD spectrum that was significantly different from the theoretical sum of the two separate componentsv indicating that the exogenous protein is capable of binding the ribonucleic acid *in vitro*. A close inspection of the CD spectra suggests transient binding of low specificity as the most likely mode of this interaction. We observed RNA preparations produced a positive signal when total RNA from *Drosophila melasnogaster* was tested with polyconal antibody generated against PPYR1. On the other hand, RNA preparations isolated from *Neurospora crassa* did not cross reactivity with the same antibody. This result was understandable because no PPYR1 homologue gene was found in the *N. crassa* genome. We assumed the PPYR1 bound to the RNA during the purification and the interaction could be abolish after RNA degradation.

Phosphorylation of PPYR1

Inside the RNA binding domain of PPYR1 we found a putative phosphorylation site for PKA. The phosphorylation of purified PPYR1 with the catalytic subunit of bovine PKA was demonstrated *in vitro*. About 1 phosphate per polypeptide chain incorporation was reached in the presence of PPYR1. The PPYR1 inhibited the activity of PPY. The protein containing one phosphate per polypeptide chain inhibited the PPY activity twice as much efficiently as the non-phosphorylated counterpart. From the K_d of the PPY-PPYR1 complex formation one can calculate that about ten times less PPYR1 should be sufficient to achieve half maximal saturation of the phosphatase that we used for the measurement. Consequently, it is likely that PPYR1 influences the enzyme activity by modifying the conformation of the phosphosubstrate. We additionally demonstrated that PPY could dephosphorylate PPYR1 protein.

Gene expression of PPYR1

In order to detect PPYR1 mRNA we used the full length cDNA of the codig region as a probe for Northern hybridization. We detected a 1.7 kb *PPYR1* transcript in the testes and a smaller 1.4 kb one in the ovaries, no *PPYR1* mRNA was found in early embryos. The expression pattern of PPYR1 mRNA was studied by *in situ* hybridizations. In accord with the Northern blot results the PPYR1 transcript was not found in early stage 2 embryos. It was also missing from late, stage 17 embryos, thus *de novo* synthesis of the protein in the embryos must be negligible. On the other hand, strong staining was observed in the early egg chambers, both in the somatic and in the germline cells. PPYR1 mRNA accumulated in the small follicular cells and in the nurse cells of the egg chamber. The mRNA was deposited into the oocyte during the rapid nurse cell cytoplasm transport, our result demonstrates the entry of the PPYR1 transcript into the stage 11 oocyte. The PPYR1 mRNA probably degrades during the process of oocyte maturation because it was not seen in the early embryos. The mRNA *in situ* hybridization gave intensive staining in the cytoplasm of the primary spermatocytes indicating the presence of PPYR1 transcript in these cells.

Localization of PPYR1 protein

To study the expression of PPYR1 during development, a specific polyclonal antibody was raised against the full length protein expressed in bacteria and was used for Western blotting. Strong immunostaining of a 47 kDa band was observed in early (stage 2) embryos. The intensity of the staining decreased in the late (stage 17) embryos. No signal was detected in blots of extracts from any of the larval stages, from pupae or from adult flies. The tissue specific expression of PPYR1 in adult flies was investigated in the reproductive organs. The protein was detected in the extracts of ovaries and testes while little or no signal was found in the carcasses devoid of the reproductive organs. The apparent molecular mass of the

immunoreactive band was 47 kDa and 66 kDa in the ovary and the testis extracts, respectively. Thus PPYR1 has a dual appearance during *Drosophila* development; it is expressed maternally as well as zygotically. In agreement with the Western blots, we detected a longer *PPYR1* transcript in the testes and a smaller one in the ovaries by Northern blotting.

The cellular localization of PPYR1 was studied by immunohistochemistry in Drosophila embryo, ovary and testis. First we investigated the distribution of the maternally expressed gene product in embryos and ovary. As expected from the Western blot results, an evenly distributed strong signal was seen in early embryos. Staining was considerably reduced in late embryos, where it was confined to the gut. Up to stage 7 the cytoplasm of follicular and germ cells exhibited intensive staining. In later stages, the expression level of PPYR1 remained high in the follicular and nurse cells, while it decreased gradually in the oocyte; it was barely detectable at stage 9, and unexpectedly, we found no signal in stage 11 oocyte at lower magnification. At higher magnification a very faint staining was visible in this oocyte close to the entry point of the nurse cell cytoplasm indicating that PPYR1 is transported to the oocyte where it becomes hardly detectable by traditional immunostaining. However, the prediction that PPYR1 is an RNA binding protein helped to resolve the discrepancy. When we treated the fixed egg chambers with RNase prior to immunohistochemistry in order to eliminate the RNAs from the samples, PPYR1 was readily detected in stage 11 oocytes at both lower and higher magnifications verifying that this protein was indeed deposited in the oocyte via the rapid nurse cell cytoplasm transport. Our findings also suggest that PPYR1 forms complexes with RNAs which effectively mask its epitopes. The Western blot experiment indicated that the zygotic expression of PPYR1 is specifically confined to the testis where PPY is also exclusively expressed. Its expression pattern supports the relevance of this interaction. According to our data both proteins are present in the germ cells and the spermatocytes within the testis of male flies. Although the majority of PPY accumulates in the nucleus, while PPYR1 resides in the cytosolic compartment they can be temporarily bound to each other in the cytosol.

5. SUMMARY

PPY and PPN are two novel Drosophila specific protein phosphatases. Although the genes and gene products of the two phosphatases have been described their physiological role remained an open question. The lack of information on the interacting partners of the phosphatases would suggest a physiological role for these enzymes. In our preliminary experiments we identified five proteins that interact with PPY and one interacting protein of PPN. In our subsequent work we selected one of the PPY interacting proteins, termed PPYR1, for a more detailed investigation. The specific interaction between PPY and PPYR1 has been confirmed by several independent methods including immunoprecipitation, "pull-down" experiment, and surface plasmon resonance spectroscopy. Based on its abnormal mobility in SDS-PAGE, CD-spectrum, sensitivity to proteases and heat stability, we concluded that PPYR1 was an intrinsically unstructured protein. The primary structure of PPYR1 has shown 40 % homology with that of PAI-1, an mRNA-binding protein. We demonstrated the RNA-binding capacity of PPYR1 by *in vitro* experiments. We found a protein kinase A recognition site in the RNA-binding region. We confirmed that protein kinase A indeed phosphorylates recombinant PPYR1 under *in vitro* conditions. We found that neither the phosphorylated, nor the dephosphorylated form of PPYR1 acted as an efficient inhibitor of the PPY phosphatase. According to its biochemical chemical properties PPYR1 can function as a scaffold for the organization of protein and RNA complexes with PPY.

The mRNA of PPYR1 was detected in the testis and ovarium of the adult fruit flies. In addition, PPYR1 protein was found in the early Drosophila embryos. The transport of PPYR1 from the nurse cells to the oocyte in the egg chamber was proven by immunohystochemical methods. It is likely that the protein of maternal origin accumulates in the embryos. We suggest that RNA-bound PPYR1 is important in the early development of the embryo. In the testis of Drosophila we observed a zygotic form of PPYR1 that has a larger molecular mass than the maternal gene product. PPYR1 and PPY proteins were localized in the small germ cells and in the early spermatocytes at the apical tip of the testis. The co-localisation of the two proteins in the same cell types makes their *in vivo* interaction possible. According to our experimental results PPYR1 has two different forms; the maternal and zygotic proteins which play different roles in the development of sperm cells or the embryos.

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7. LIST OF PUBLICATIONS

This thesis based on the following publications:

E. Kókai, Á. Tantos, E. Vissi, B. Szöőr, P Tompa, J. Gausz, L. Alphey, P. Friedrich, V. Dombrádi: CG15031/PPYR1 is a gonad specific intrinsically unstructured protein that interacts with protein phosphatase Y.
Arch Biochem Biophys.; 1;451(1):59-67 (2006) IF: 3,152

E. Kókai, M. Szuperák, L. Alphey, J. Gausz, G. Ádám, V. Dombrádi: Germ line specific expression of a protein phosphatase Y interacting protein (PPYR1) in Drosophila.
Gene Expr Patterns.; (7):724-9. (2006)
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T. Zeke, B. Szöőr, **E. Kókai**, E. Yatzkan, O. Yarden, K. Szirák, Z. Fehér, P. Gergely and V. Dombrádi: Analysis of protein phosphatase 1 gene expression in *Neurospora crassa*.
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E. Kókai, L. Alphey, V. Dombrádi: Functional analysis of PPY and PPN testis specific protein phosphatases of *Drosophila melanogaster.*
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