FUNCTIONAL ANALYSIS OF NOVEL PROTEIN PHOSPHATASES IN *DROSOPHILA MELANOGASTER*

Endre Kókai

UNIVERSITY OF DEBRECEN, MEDICAL AND HEALTH SCIENCES CENTER
DEPARTMENT OF MEDICAL CHEMISTRY

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 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 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 nore 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 colocalisation 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.