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Germ line specific expression of a protein phosphatase Y interacting protein (PPYR1) in *Drosophila*

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Abstract

PPYR1, the product of the *CG15031* gene, was identified as a protein phosphatase Y (PPY) interacting protein in *Drosophila melanogaster* using a yeast two-hybrid screen. PPYR1 displays a biphasic expression pattern: the maternal protein is abundant in the developing egg chambers and in the early embryos, while the zygotic protein appears later in development and is localized specifically in the testes of the males. The maternal and zygotic gene products differ from each other in their size having apparent molecular masses of 47 and 66 kDa, respectively. The maternal PPYR1 is localized in the cytoplasm of the follicular and nurse cells and is deposited as a ribonucleoprotein complex in the oocyte. In the early embryos, the PPYR1 is distributed evenly, and it gradually diminishes during embryonic development. Zygotic PPYR1 is expressed exclusively in the testes, predominantly in the cytoplasm of the spermatocytes. PPY is localized in the nuclei of the same cells. Our results suggest that PPYR1 has two distinct developmental isoforms: a maternal protein the expression of which is independent of PPY and a zygotic protein which is co-expressed with PPY.

Keywords: *CG15031*; Protein phosphatase Y interacting protein; Spermatocyte; Oocyte; Subcellular localization; *Drosophila melanogaster*

1. Results and discussion

Phosphoprotein phosphatases are key regulators of protein phosphorylation based signal transduction in all eukaryotic organisms; their hydrolytic enzyme activity renders the regulatory mechanism reversible. After sequencing the euchromatin of *Drosophila* 16 genes coding for the catalytic subunits of protein phosphatases of the PPP enzyme family were identified (Morrison et al., 2000; Kókai et al., 2001). Among them, protein phosphatase Y (PPY) is a unique, sex specific member of the family, the expression of which is limited to the *Drosophila* testes. Its cDNA was cloned from *Drosophila melanogaster* and its gene (termed *PpY-55A/CG10930*) was localized to the second chromosome at 55A2 (Dombrádi et al., 1989; Foehr and Doane, 1994). A 1.4 kb PPY mRNA is synthesized specifically in the testis of third instar larvae, pupae and adults. The PPY protein was reported to accumulate in the nuclei of cyst cells (Armstrong et al., 1995). Based on its expression pattern, the involvement of PPY in the development of sperm cells was suggested. Recently, we identified several interacting partners of PPY using the yeast two-hybrid method (Kókai et al., unpublished results). One of the interacting proteins, termed protein phosphatase Y regulator 1 (PPYR1), is encoded by the *CG15031* gene. *CG15031* was sequenced by the Berkeley *Drosophila* Genome Project (http://flybase.bio.indiana.edu/, FlyBase Genome Annotators, 2004 Release 3.2 of the annotated *Drosophila melanogaster* genome) and was mapped to

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13B3 on the X-chromosome. Based on the genomic and cDNA sequences, a protein of 309 amino acids containing a hialuronan/mRNA binding domain has been suggested as the only gene product (Heaton et al., 2001). The biochemical characterization of PPYR1 and the demonstration of its interaction with PPY will be described elsewhere; in this communication we present its specific cellular distribution and subcellular localization in *Drosophila*.

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 (Fig. 1A). 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. Faint bands were observed in extracts from pupae and adults when the sensitivity of detection system was elevated by increasing the concentrations of both the first and second antibodies (data not shown). The tissue specific expression of PPYR1 in adult flies was further investigated (Fig. 1B). 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 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. The zygotic protein has a lower relative mobility than the maternal one, and its expression is restricted to the testis. Differential phosphorylation of the protein can not explain the mobility shift as the non-phosphorylated and phosphorylated forms of the protein have the same relative molecular mass values (Kókai E. unpublished result). In agreement with the Western blots, we detected a 1.7 kb *PPYR1* transcript in the testes and a smaller 1.4 kb one in the ovaries by Northern blotting (Fig. 2). Interestingly, no *PPYR1* mRNA was found in early embryos where the protein was most abundant, suggesting that the PPYR1 in the embryos has a maternal origin.

The cellular localization of PPYR1 was studied by immunohistochemistry. First, we investigated the distribution of the maternally expressed gene product. As expected

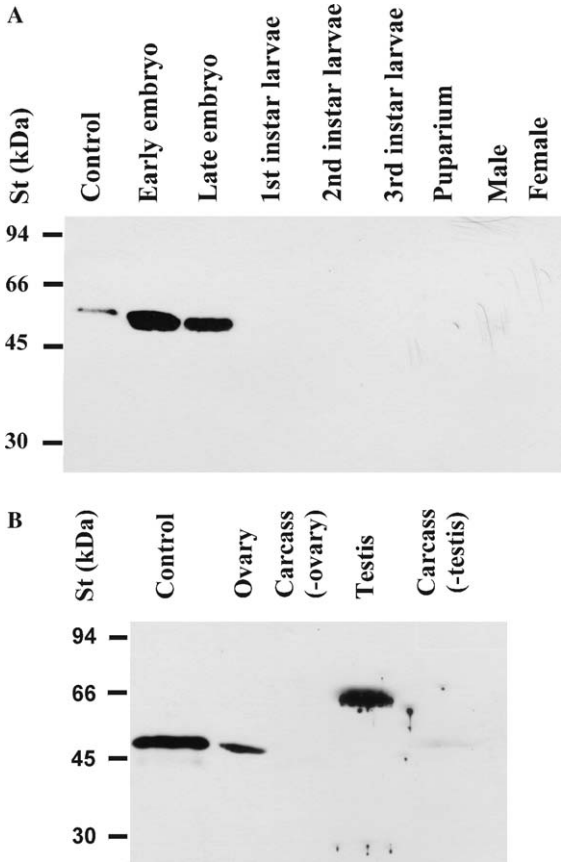

Fig. 1. Expression of PPYR1 protein in *Drosophila*. (A) The expression of PPYR1 during different developmental stages of *Drosophila* was investigated by Western blotting. Crude extracts (30 μg protein) of early (stage 2) embryos, late (stage 17) embryos, first, second, and third instar larvae, pupae as well as male and female flies were separated by SDS–PAGE and tested with a PPYR1 specific antibody. Recombinant His-PPYR1 was used as a positive control. (B) The tissue specific expression of PPYR1 was studied in adult *Drosophila*. Ovaries were isolated from five female flies, and testes were dissected from five male adults. The remaining carcasses were also analyzed by Western blotting. St denotes molecular mass standards.

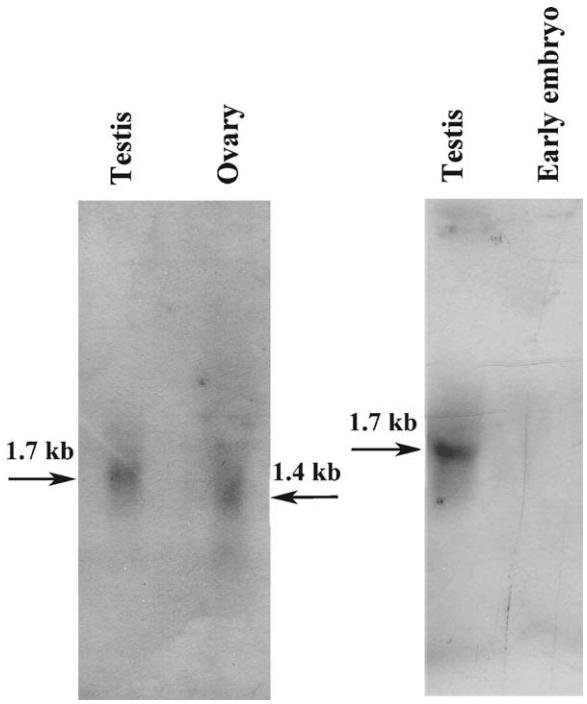

Fig. 2. Tissue specific forms of the PPYR1 transcript. Thirty micrograms of testicular RNA, 70 μg ovarian RNA, and 30 μg RNA isolated from early (stage 2) embryos were analyzed by Northern blotting with a PPYR1 specific cDNA probe. The bands were visualized by autoradiography after 7 days of exposure, and their sizes were estimated relative to the three rRNA bands.