Identification and characterisation of *poirot* gene in *Drosophila melanogaster*

Summary of Ph.D.thesis

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Introduction

The embryonic polarity of many organisms is defined by maternally provided determinants that are localised in the oocyte during egg development. Localisation and the subsequent site-specific translation of mRNAs provides one of the mechanisms that restrict the biological functions of determinants to specific cytoplasmic regions within the oocytes. This type of regulation has been studied most extensively in *Drosophila melanogaster,* since the antero-posterior and the dorso-ventral embryonic body axes, and germ line differentiation is governed by maternally provided localised determinants. In many organisms the germ-cell determinants, germ plasm, are localised to an identifiable part of the cytoplasm of the developing embryo (Beams and Kessel, 1974; Eddy, 1975).

The assembly of the germ plasm is interconnected with processes required for forming the anterior-posterior axis of the oocyte and for localising RNAs to specific place during oogenesis. Germ plasm contains all of the components required to determinant the germ cell fate. As a first step of germ cell formation a small subset of syncytial nuclei migrate into the germ plasm and the arriving nuclei are directed into a germ cell fate by molecules stored in the posterior cytoplasm as a collection of mitochondria, fibrils, and electrodense polar granules (Mahowald, 1962). This induce the formation of the pole cells, which are restricted to germline fate in the posterior end of the blastula *Drosophila* embryo (Hay *et al.*, 1988; Lasko and

Ashburner, 1990). The pole cells undergo a patterned migration to the embryonic gonad during the later gastrulation.

Embryonic germ cell formation and abdomen development in *Drosophila* requires localisation and site specific translation of *oskar* mRNA in the posterior part of the oocyte. Targeting of *oskar* function to the posterior pole of the oocyte needs a large set of proteins and RNAs, encoded by posterior group genes. Consequently, mutations in the posterior group genes can result in embryos without abdomens and/or germ cells. Posteriorly anchored Osk protein is a key component of the germ plasm and subsequent embryonic germ cell formation. Although, the anchoring process is an important aspect of *osk* regulation very little is known about its mechanism. Genetic analysis of the anchoring mechanism is especially difficult, since Osk anchoring is interdependent both on the presence of the mRNA and the protein at the posterior region (Markussen *et al.*, 1995). Therefore, mutations of regulatory genes that interfere with Osk protein localisation often also result in mRNA delocalisation, making it impossible to separate these two processes. An example of the above is provided by the *TmII* mutation, which abolishes the localisation of both *osk* mRNA and protein (Erdelyi *et al.*, 1995; Tetzlaff *et al.*, 1996). Therefore, the actin binding TmII protein and the actin cytoskeleton may be directly connected, not only to the mRNA but also the Osk protein, at the posterior pole.

Objectives

The objective of our laboratory to identify new factors that have a role in germ plasm assembly and the embryonic germ cell formation in *Drosophila melanogaster*.

The main goal was to isolate novel posterior genes. We decided to use a *hobo* mutator element, which has a pattern of insertion different from that of the commonly used P elements, and allows the rapid cloning of the tagged genes.

We screened a non-lethal, autosomal, *hobo* induced mutant collection, and isolated prt^{gs} as a new grandchildless mutation.

Our aim was to characterise the new prt^{gs} mutation and *prt* gene by genetic and molecular biology methods. We wanted to understand the *prt* function in the posterior gene hierarchy.

Methods

- Fly strains and genetics
- Hobo element remobilisation
- Embryonic cuticle preparation
- Cloning by adapter PCR strategy
- pUAS-prt and pUAS-prt-GFP rescue construct generation
- Total RNA purification and Northern hybridisation
- RT-PCR

- Generation of the PRT polyclonal antibody by GST-Prt fusion protein
- Western blot analysis
- Detection of proteins in whole mount in ovary and embyo
- RNA in situ hybridisation by Dig labelled DNA and RNA

Results

- We screened 750 viable autosomal *hobo* insertion lines and we found the prt^{gs} line by 70% granchildless (gs) phenotype
- We found that null mutant of *prt* produces embryos that display a significant reduction in the number of germ cells.
- The prt^{gs} *hobo* insertion was mapped to the 51 D6-12 chromosomal region by in situ hybridisation and by uncovering deficiencies
- We remobilised the insertion from the *prt* locus and five revertants were isolated by the complete loss of the grandchildless phenotype.
- We clone the *prt* gene and PRT protein shows extended homology to *Sab*, a human SH3 domain binding protein
- We show the intronic *hobo* element insertion resulted in RNA null *prt* allele by forcing an aberrant splicing
- We found the PRT protein in large protein aggregates that are localized subcortically in the oocytes.

- We show a reduction of short Osk protein levels and abnormal Osk distribution.
- We showed that *prt*^{gs} and the BTK29A mutations interact genetically.
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Discussion

In *prt*^{gs} mutants the Osk protein delocalisation does not seem to be coupled with *osk* mRNA delocalization. While *osk* mRNA was localised normally at the posterior pole in all stages of oogenesis, some Osk protein was detached from the subcortical region in *prt*^{gs}/*prt*^{gs} mutant ovaries. This delocalisation can reduce Osk concentration significantly at the posterior pole. The decreased Osk level leads to germ plasm reduction and the formation of fewer pole cells, which explain the *prt* mutant phenotype. As in early stages of oogenesis Osk localisation seems completely normal and only becomes abnormal gradually during the subsequent stages, *prt* is very unlikely to interfere with Osk translation, rather it plays a role in Osk protein anchoring at the posterior pole or increases the stability of the anchored Osk protein.

In *prt*^{gs} mutants only the short Osk isoform level was found to be reduced while the long isoform remained at wild type levels. This may explain the normal Osk mRNA distribution, since the long Osk isoform can maintain its own mRNA at the posterior pole as described earlier (Markussen *et al.*, 1995). However, the isoform specificity of

prt^{gs} reveals that the two Osk isoforms may utilise independent posterior anchoring mechanisms or can be subjected to different post-translational regulation processes during their anchoring. On the other hand genetic and molecular evidences suggest that some residual short Osk must still be present in *prt*^{gs} null mutants, because the complete loss of the short Osk isoform would not only result in grandchildless but rather a complete abdomen and germ cell-less phenotype. This result also demonstrates that *prt* function in Osk anchoring must be redundant.

Subcellular localisation of PRT is reconcilable with *osk* regulatory function, since at the subcortical region their localisation overlaps. This colocalisation potentiates the functional interaction. Some *osk* translational regulatory proteins, which form a large ribonucleoprotein (RNP) complex, demonstrate a similar expression pattern during their transport to oocytes to that of PRT. Biochemical evidence exists that this RNP contains EXU and at least seven other proteins in addition to *osk* and *bicoid* mRNAs (Wilsch-Brauninger *et al.*, 1997; Wilhelm *et al.*, 2000). We demonstrated that Exu, Orb, and Me31, three elements of the above RNP complex, as well as CUP, an RNP independent protein with similar localisation pattern (Keyes and Spradling, 1997), are not colocalised with PRT in nurse cells. Consequently PRT aggregates, in spite of their similar subcellular localisation, have an independent transport system to either the EXU or the CUP complexes.

The genetic interaction found between *prt* and *BTK29A* indicates that the the PRT regulatory function is evolutionary conserved. This is especially interesting, since the Drosophila BTK homologue is not required in germ cell formation(Baba *et al.*, 1999). However, our germ line clone analysis of the two hypomorhic alleles failed to reveal a role in germ cell formation. The Drosophila genome contains a single BTK homologue *Btk29A*, which encodes for two protein species. *Btk29A* has several pleiotropic functions such as male fertility and ring canal formation. It is expressed in the adult head, the larval immune system, the male and female gonads and several other tissues (Vincent, *et al.*, 1989; Wadsworth *et al.*, 1990).

Based on the structural conservation of the Sab and PRT proteins, we anticipated that PRT might also exhibit a negative regulatory function similarly to Sab. According to this hypothesis, when PRT, the presumed negative regulator is absent, an ectopic BTK activity would interfere with the normal function of the posterior gene hierarchy. Being normally suppressed, loss of function of such a negatively regulated gene would not be expected to cause any posterior phenotype. Indeed, the suppression of prt^{gs} phenotypes by hypomorphic *Btk29A* alleles indicates that in Drosophila, also PRT negatively regulates BTK29A.

Therefore we propose that, in wild type oocytes, PRT inactivates the BTK protein in the subcortical region. In prt^{gs} mutants however, unregulated BTK interferes either with the localisation of subcortical cellular components in the oocyte, or it modifies the phosphorylation

pattern of the short Osk protein itself. We find that the latter is a less feasible explanation, because in *prt*^{gs} mutants we do not find increased levels of phosphorylated short Osk that would be the result of extra kinase activity, instead the level of 57 kDa phosphorylated short Osk isoform is also significantly reduced. We suggest that uncontrolled BTK kinase activity modifies the anchoring capability of short Osk directly or indirectly to the subcortical region, resulting in delocalisation and degradation of both phosphorylated and unphosphorylated short Osk proteins. Since only the phosphorylated short Osk isoform was detected in *prt*^{gs} mutants, we suppose that either this protein is more stable or it is better anchored to the posterior pole, compared to the unphosphorylated one. The weakly anchored Osk protein is displaced by the cytoplasmic streaming and loses its pole plasm organizing activity.

Future research should be directed towards determining the precise biochemical function of PRT and elucidating the anchoring mechanism of Osk protein.

Publication

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