Developing novel methods to identify genes involved in germ line induction of *Drosophila melanogaster*

Ph.D. thesis

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Introduction

Embryonic germ cell development in *Drosophila melanogaster* depends on the germ plasm, the most posterior part of the ooplasm. There is experimental evidence that the germ plasm contains all the factors necessary for the formation of the embryonic germ cells. Though most of the germ plasm components have not been identified yet, it is known that at least in part the germ line factors are stored in the form of RNA. Besides factors that make the difference between the germ cell and the somatic cell fate, pre-localized germ plasm might also contain gene products that govern early development of the germ cells since their own genome becomes transcriptionally active only during the gastrulation stage when the germ cells move into the midgut invagination. In addition, determinants needed for the proper development of the posterior part of the soma are also localized in the germ plasm.

Assembly of the germ plasm is a stepwise process. First, during mid-oogenesis a founder molecule, the *oskar* (*osk*) mRNA, is tightly localized to the posterior pole where it gets translated. Then the posterior-restricted Osk protein recruits the other germ plasm components. The focus of germ cell formation depends exclusively on the site of the *osk* RNA localization since the anterior mislocalized *osk* mRNA results in anterior germ cells. A group of genes, collectively called posterior group genes, have been identified by their somatic mutant phenotype similar to that of *osk* loss of function mutations.

The aim of my work was to establish a method to identify novel germ plasm componets on a genome scale level. To achieve our goals, we combined the cDNA microarray method with a genetic interaction type of assay.

Materials and methods

Microarray analysis

Digoxigenin (DIG) labeling of RNA and DNA molecules

RNA *in situ* hybridization on embryos

Establishment and testing of the *staufen oskar TropomyosinII* (SOT) sensitized background

Molecular biological techniques (genomic DNA purification, cloning the insertion point of the P-elements with inverse PCR)

Bioinformatics (sequence analysis)

Discussion

In the cDNA microarray experiments, we compared deficient, normal and ectopic germ plasm conditions. We assumed that it was possible to affect the concentration of germ plasm specific RNAs by germ plasm mutations if the stability of such RNAs was dependent on the level and the existence of the germ plasm. In our experiments, we tried to measure differences in the stability of pole plasm specific RNAs. To do so, we performed transcript profiling of seven conditions in which the level of the germ plasm was genetically modified. Sixty RNA species out of 3200 were selected as exhibiting the expected microarray pattern. We investigated their distribution in wild type and ectopic germ plasm containing embryos using the *in situ* RNA hybridization technique. We found that seventeen out of sixty showed germ plasm specific localization.

For the functional analysis of the localized RNAs and in order to identify additional germ plasm specific RNAs, we

established a genetic interaction type of assay. The assay is based on a *Drosophila* strain that carries three mutations of posterior group genes in heterozygous form resulting in a moderate germ cell deficient phenotype. Using this sensitized genetic background, we performed a screen on a third chromosomal P element insertion collection. 600 P element insertions were analyzed and 26 of them proved to be significant enhancers of the germ cell deficient phenotype. The sites of the P element insertions in these 26 lines were molecularly mapped and the affected genes were identified.

We arranged the newly identified genes into a network based on their earlier established protein-protein interactions. Besides the novel genes, this network contains all the posterior group genes which are linked to them with maximum one linker gene.

Our results indicate that we successfully developed elements of a reverse genetic experimental system which combines a genetic interaction assay with the cDNA microarray technique, and that it is applicable on a genome scale level.

Scientific publications:

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Kókai E., **Szuperák M**., Alphey L., Gausz J., Ádám G., Dombrádi V.
Germ Line Specific Expression of a Protein Phosphatase Y Interacting Protein (PPYR1) in *Drosophila*.
Gene Expression Patterns. Elfogadva 2005 dec. 21.

Szuperák M.
Developing a novel method to identify genes involved in germ line induction of *Drosophila melanogaster* embryos.
(dissertation summary)
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