Developing a novel method to identify genes involved in germ line induction of *Drosophila melanogaster* embryos

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Embryonic germ cell development in *Drosophila melano*gaster depends on the germ plasm, the most posterior part of the ooplasm. There are experimental evidences that germ plasm contains all the factors necessary for the formation of the embryonic germ cells (Mahowald 2001). 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 (Tomancak et al. 2002). The aim of our work was to identify novel germ plasm-enriched, localized RNAs by combining cDNA microarray and RNA *in situ* hybridization techniques and to verify the results with a functional analysis.

In the cDNA microarray experiments we compared deficient, normal and ectopic germ plasm conditions using a chip containing 3200 cDNAs of annotated *Drosophila* genes. Sixty RNA species were selected as exhibiting the expected microarray pattern and subsequently investigated on their distribution in wild type and ectopic germ plasm containing embryos. We found that 17 out of 60 showed germ plasm specific localization (Szuperák et al. 2005).

For the functional analysis of the localised RNAs and to identify additional germ plasm specific RNAs we have established a genetic interaction type of assay. This assay is based on a strain that carries three mutations 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 such mutations were analysed and 26 of them were significant enhancers of the germ cell deficient phenotype. The site of the P-element insertions in these 26 lines were molecularly mapped and the affected genes were identified by BLAST search. The BLAST searches revealed that one of the 26 lines were selected previously in the microarray experiment and some of them turned out to be alleles of genes whose function in the germ line development has been already established.

These results indicate that we have developed a reverse genetic experimental system which in combination with the genetic interaction assay enables the isolation at genom scale level and the analysis of new factors involved in Drosophila germ line differentiation.

References

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