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# Identification of germ plasm-enriched mRNAs in *Drosophila melanogaster* by the cDNA microarray technique

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**Abstract**

The development of embryonic germ cells in *Drosophila* depends on the germ plasm, the most posterior part of the ooplasm. The germ plasm is devoted to the formation of future germ cells and is known to contain all the factors that are necessary to induce germ cell fate. Besides having a characteristic organelle and protein distribution, the germ plasm also contains a large number of localized RNA species that have been shown to play crucial roles in germ cell determination. To identify germ plasm-enriched, localized transcripts, we used a two-step method composed of cDNA microarray (containing 3200 annotated *Drosophila* cDNAs) and in situ RNA hybridization techniques. We compared germ plasm deficient, normal and ectopic germ plasm conditions in the cDNA microarray experiments. RNA species whose concentration increased when ectopic germ plasm was present and decreased when the germ plasm was missing were selected. These candidates were then subjected to a second screen which compared the distribution of the given RNA in wild type embryos and in eggs with ectopic germ plasm. Finally, 17 RNA species were found to be enriched in the germ plasm. Based on these data, we estimate that around 1% of the *Drosophila* genes encode for germ plasm-enriched, localized transcripts. We conclude that this combination of microarray and in situ hybridization techniques is a simple but powerful experimental design for the genome-wide identification of genes coding for germ plasm localized transcripts.


*Keywords:* Germ cells; Germ plasm; Pole plasm; Gene chip; DNA microarray; In situ hybridization; *Drosophila melanogaster*; RNA localization; RNA decay; RNA stability; Oskar

## 1. Results and discussion

Early *Drosophila* development takes place in a syncytium in which rapidly dividing nuclei of the early embryo are nested in the common cytoplasm of the egg resulting in a multinucleate cell. Early syncytial nuclei are transcriptionally quiescent and totipotent (Zalokar, 1971, 1976). Developmental events including division, movement of the nuclei to the periphery and their primary determination are controlled by maternal factors. When reaching the cortex, the nuclei come into contact with maternally inherited pre-localized positional signals. These signals determine both the antero-posterior and dorso-ventral axes of the differentiating embryo as well as the place of germ cell differentiation (Riechmann and Ephrussi, 2001). The signals responsible for germ cell differentiation are localized at the most posterior part of the egg and contain factors that induce the germ line fate of those nuclei that reach the cortex and cellularize at this particular place (Mahowald, 2001). 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 the 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 (Zalokar, 1976). In addition, determinants that are needed for the proper development of the posterior part of the soma also localize in the germ plasm.

Assembly of the germ plasm is a stepwise process (reviewed in Riechmann and Ephrussi, 2001). First, during mid-oogenesis a founder molecule, the *oskar* (*osk*) mRNA, is tightly localized to the posterior pole where it gets

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translated. Then the posterior restricted Osk protein recruits other germ plasm components. The focus of germ cell formation depends exclusively on the site of the *osk* RNA localization since anterior mislocalized *osk* mRNA results in anterior germ cells (Ephrussi and Lehmann, 1992). Moreover, it has been shown that the number of the germ cells at the posterior pole also depends on the concentration of the localized *osk* transcript (Ephrussi and Lehmann, 1992; Smith et al., 1992). 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 (St Johnston and Nusslein-Volhard, 1992). Via the systematic analysis of the posterior group genes, a limited number of germ plasm components have been identified in the early 1990s such as, the Staufen, Tudor and Vasa proteins and the *nanos*, *spire* and *pumilio* RNAs (St Johnston et al., 1991; Wang and Lehmann, 1991; Bardsley et al., 1993; Barker et al., 1992). In parallel experiments targeting other aspects of early *Drosophila* development, additional germ plasm localized RNAs have been described: *CylinB*, *germ cell-less*, *mtlrRNS* (Raff et al., 1990; Jongens et al., 1992; Kobayashi et al., 1993). Later, a number of germ plasm-enriched transcripts were found in differential cDNA screens, in which RNA content of the anterior and posterior halves of early embryos (Ding and Lipshitz, 1993) or germ plasm deficient and normal embryos were compared (Nakamura et al., 1996). Recently, about 30 germ plasm-enriched RNAs have been discovered during an ongoing systematic RNA in situ hybridization experiment of the Berkeley *Drosophila* Genome Project (BDGP) (Tomancak et al., 2002) which raised the total number of the known germ plasm-enriched RNAs up to 40. To isolate novel germ plasm-enriched mRNAs, we carried out a cDNA chip screen on cDNAs prepared from germ plasm deficient developing eggs and from those that contained ectopic germ plasm.

### *1.1. Genetic manipulation of the level of germ plasm in developing oocytes*

Germ plasm is restricted to a small part of the oocyte, therefore, collecting a reasonable volume from it for the DNA chip analysis is difficult. However, a number of mutations are available by which genetic manipulation of the level of the localized *osk* RNA and therefore the level of germ plasm can be easily accomplished. For this purpose, we chose mutant alleles of the cytoskeletal gene *Tropomyosin II* (*TmII*) which is known to interfere with the accumulation of the *osk* mRNA at the posterior pole (Erdelyi et al., 1995; Tetzlaff et al., 1996). In *TmII* mutant eggs, the level of the Osk protein is strongly reduced and as a consequence germ cell-less progeny develop. Among the progeny of $TmII^{gs1}$, $TmII^{eg9}$, and $TmII^{eg20}$ homozygous females, we observed 98.4, 96.3 and 89.7% occurrence of germ cell less individuals, respectively. To completely eliminate germ plasm from the developing oocytes, we applied a protein null *osk* allele combination. Embryos from $osk^{A87}/osk^{54}$ heterozygous females showed 100% penetrance of maternal effect lethal phenotype with posterior somatic defects characteristic of the *osk* null mutants (Lehmann and Nusslein-Volhard, 1986). To increase the amount of the germ plasm, we used the gain of function *osk–bcd3′UTR* transgene (Ephrussi and Lehmann, 1992). In this transgene, the *osk* coding region is fused to the 3′ localizing signal of the *bicoid* (*bcd*) mRNA (*osk–bcd3′UTR*) (Macdonald and Struhl, 1988) which directs the chimeric mRNA to the anterior pole of the egg. We found anterior localized *osk* mRNA in 100% of the eggs from *osk–bcd3′UTR* heterozygous mothers while the *osk* transcript was also present at the posterior pole where it normally occurs. We also observed in this experiment that ectopic *osk* mRNA resulted in a fully penetrant so called bicaudal maternal effect lethal phenotype as was originally described by Ephrussi and Lehmann (1992).

### *1.2. Measurement of RNA stability in the Drosophila oocyte with cDNA microarray*

*Drosophila* oocytes develop in cysts in which 15 nurse cells produce all the materials for the single oocyte (for detailed description see de Cuevas et al., 1997). Products of the nurse cells are transported into the oocyte through cytoplasmic bridges where they either govern development of the oocyte itself or remain stored during embryonic development. Since the transcription of the germ plasm specific RNAs and their accumulation in the germ plasm takes place in different cells, genetic manipulation of the germ plasm level most probably has no effect on their transcription. Nevertheless, 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 existence of the germ plasm. There are examples of microarray experiments in which the stability of RNAs was successfully measured in *Escherichia coli* and *Saccharomyces cerevisiae* after a transcription block (Bernstein et al., 2002; Selinger et al., 2003; Grigull et al., 2004; Wang et al., 2002). 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.

#### *1.2.1. Sample generation*

We lowered the level of the germ plasm using three *TmII* mutations: *gs1*, *eg9* and *eg20*. Since the *eg9* and *eg20* alleles are derivatives of *gs1*, they may contain identical sets of background mutations (Erdelyi et al., 1995). To control the undesirable background effects, we also performed a transcript profiling of a phenotypic revertant allele of the *gs1* mutation (named *gs1ex1*) which showed no germ plasm defect but contained the same genetic background. All *TmII* mutations were used in homozygous state, and we refer to