

Precocious Expression of *Drosophila Rbp9* Inhibits Ovarian Germ Cell Proliferation

Kyoungsuk Jeong and Jeongsil Kim-Ha

Department of Molecular Biology, School of Natural Sciences, Sejong University

The *Drosophila* RNA binding protein RBP9 and its *Drosophila* and human homologs, ELAV and the Hu family of proteins, respectively are highly expressed in the neuronal cells. Despite the predominant expression of RBP9 in nerve cells, mutational analysis revealed a female sterile phenotype for *Rbp9* mutants. We further showed that RBP9 is expressed not only in the nuclei of neuronal cells, but also in the cytoplasm of cystocytes during oogenesis. The female sterility phenotype of the *Rbp9* mutants resulted from defects in oogenesis; the lack of *Rbp9* activity caused the germarium region of the mutants to be filled with undifferentiated cystocytes. RBP9 appears to stimulate cystocyte differentiation by regulating the expression of *bag-of-marbles* (*bam*) mRNA, which encodes a developmental regulator of germ cells. RBP9 protein bound specifically to *bam* mRNA *in vitro*, which is required for cystocyte proliferation, and the number of cells that expressed BAM protein was increased five- to tenfold in the germarium regions of *Rbp9* mutants. These results suggest that RBP9 protein binds to *bam* mRNA to down regulate BAM protein expression, which is essential for the initiation of cystocyte differentiation into functional egg chambers.

To further examine the role of *Rbp9* in germ cell proliferation regulation, we over-expressed it ectopically in germline stem cells and early stage cystocytes that had not yet formed 16 cell clusters. The egg chambers of the newly eclosed transgenic flies looked normal. However, as time went by the number of egg chambers in each ovariole decreased. On day 12, most of the developing egg chambers had disappeared and only the germarium region remained. Staining of the *Rbp9* over-expressing transgenic ovaries with BAM revealed almost no mitotic divisions: in contrast to the 2~10 germ cell staining pattern observed in wild type germaria, hardly any cystocytes expressing BAM protein were seen in the transgenic germaria. To examine these cystocytes, the ovaries were stained with HTS [hu-li tai shao protein], which labels the fusome structure. This revealed that the cells in the germarium region consisted of stem cells and cystocytes that had not yet completed four mitotic divisions. These observations suggest that precocious expression of *Rbp9* inhibits cystocyte proliferation. Therefore the precise onset of *Rbp9* expression in germarium region 2a is critical for inhibiting their further proliferation and achieving their correct differentiation.