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# *Drosophila* tensin plays an essential role in cell migration and planar polarity formation during oogenesis by mediating integrin-dependent extracellular signals to actin organization

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ABSTRACT

Oogenesis in *Drosophila* involves very dynamic cellular changes such as cell migration and polarity formation inside an ovary during short period. Previous studies identified a number of membrane-bound receptors directly receiving certain types of extracellular inputs as well as intracellular signalings to be involved in the regulation of these dynamic cellular changes. However, yet our understanding on exactly how these receptor-mediated extracellular inputs lead to dynamic cellular changes remains largely unclear. Here, we identified *Drosophila* tensin encoded by *blistery* (*by*) as a novel regulator of cell migration and planar polarity formation and characterized the genetic interaction between tensin and integrin during oogenesis. Eggs from *by* mutant showed decreased hatching rate and morphological abnormality, a round-shape, compared to the wild-type eggs. Further analyses revealed that obvious cellular defects such as defective border cell migration and planar polarity formation might be primarily associated with the decreased hatching rate and the round-shape phenotype of *by* mutant eggs, respectively. Moreover, *by* mutation also induced marked defects in F-actin organization closely associated with both cell migration and planar polarity formation during oogenesis of *Drosophila*. Notably, all these defective phenotypes observed in *by* mutant eggs became much severer by reduced level of integrin, indicative of a close functional association between integrin and tensin during oogenesis. Collectively, our findings suggest that tensin acts as a crucial regulator of dynamic cellular changes during oogenesis by bridging integrin-dependent extracellular signals to intracellular cytoskeletal organization.



## 1. Introduction

Oogenesis is accompanied by many dynamic cellular changes, such as stem cell differentiation, dynamic cell cycle control, cell polarization, cytoskeletal polarization, active transport of materials between cells, and cell migration. *Drosophila* oogenesis has been a good subject of research for obtaining mechanistic insights of these dynamic cellular changes since *Drosophila* system allows convenient genetic and *in vivo* imaging approaches [1–3]. Indeed, many genetic factors related to oogenesis have been successfully identified from *Drosophila* studies [1,4–6]. For example, an important role of the Bone Morphogenetic Protein (BMP) signaling in stem cell differentiation has been first characterized by *Drosophila* oogenesis studies [4]. Other *Drosophila* studies have revealed that Gurken (Grk), Par-1, Bazooka/Par-3, Par-6 and aPKC are important factors for cell polarity formation during oogenesis [1,5]. In addition, receptor tyrosine kinases such as EGF and PDGF/VEGF receptors have been identified as regulators of border cell migration, an obvious cell migration event, during *Drosophila* oogenesis [6]. These identified factors are categorized into two distinct groups; one includes extracellular inputs and their associated membrane-bound receptors, and the other is intracellular regulators directly controlling diverse intracellular processes. The fact that extracellular inputs and intracellular regulators are both very important for oogenesis suggests that there may exist certain combinatorial associations between them [2]. Although some of these associations such as cadherin-catenin complex have been characterized, yet larger portion of them remains uncharted [7,8].

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As one of these genetic factors, a membrane-bound receptor protein, integrin encoded by *inflated* (*if*) in *Drosophila*, has been identified [9]. Previous studies showed that follicle cell clones lacking position-specific (PS) integrin displayed defective egg phenotypes [10–12]. While the most well identified function of integrin is its mediation of cell-to-cell and cell-to-extracellular matrix (ECM) connections, several studies on integrin reported additional functions of integrin such as transcriptional regulation through its interaction with intracellular JAK-STAT or MAPK signaling pathways [13,14]. Moreover, integrin is also able to control lipid rafts by affecting activity of Rho family proteins that regulate actin cytoskeleton [15]. To perform these diverse functions, integrin is known to interact with many intracellular signaling partners [13,14]. For instance, our previous study showed that integrin regulated JNK signaling during *Drosophila* wing development, which involved *Drosophila* tensin encoded by *blistery* (*by*) [16]. However, it remains unclear whether different cellular contexts confer integrin selectivity in the choice of its interacting partners. In terms of oogenesis, although the involvement of integrin in the regulation of oogenesis has been demonstrated, molecular details are yet to be clarified more.

In the current study, we focused on the role of *Drosophila* tensin in oogenesis and showed that tensin might act as a key mediator bridging integrin-dependent extracellular inputs to intracellular cytoskeletal organization to regulate dynamic cellular changes during *Drosophila* oogenesis.

## 2. Materials and methods

### 2.1. Drosophila strains

The hypomorphic allele of αPS2 integrin, $if^3$, was obtained from the Bloomington *Drosophila* Stock Center. The *by* mutant, $by^2$ is a P-element insertion line. The other *by* mutants such as $by^{rv8}$, one of the revertants, and $by^{ex49}$, one of the imprecise excision alleles, were generated by P-element excision using $by^2$ mutant [9]. In $by^{ex49}$, 3.56-kb of genomic region including most of the *by* exon was deleted (Fig. S1A).

### 2.2. RNA in situ hybridization

RNA *in situ* hybridization experiments were performed with digoxigenin-labeled RNA probes using RT-PCR products of *by* and *oskar* as DNA templates as described previously [17].

### 2.3. Preparation of embryonic cuticles

To observe the micropyle, embryonic cuticles were prepared as described previously [18]. Briefly, embryos were collected overnight from wild-type or mutant females, and fixed in 4% paraformaldehyde in PBS for 1 h. The treated embryos were mounted in Hoyers/lactic acid, and examined under a light microscope.

### 2.4. Immunohistochemistry

Ovaries were dissected and fixed in 4% paraformaldehyde in PBS. They were then washed in PBST (PBS + 0.1% Tween20) and blocked in PBST with 3% BSA. The subjects were incubated with anti-Fasciclin III (FasIII) mouse monoclonal antibody (1:100, Developmental Studies Hybridoma Bank, University of Iowa, IA) or anti-phosphotyrosine mouse antibody (1:100; Upstate, VA). The ovaries were again washed and incubated for 4 h at room temperature in FITC-labeled anti-mouse secondary antibody (Sigma, MO) at the dilution of 1:200. In order to visualize the actin structure, the ovaries were stained with TRITC-labeled phalloidin (Sigma, MO) for 20 min. After washing, the ovaries were stained with Hoechst 33258 (Sigma, MO) for an additional 10 min, and then were washed and analyzed using a LSM510 laser confocal microscope (Carl Zeiss, Germany). Basal actin structures of follicle cells were examined as described previously [19].

## 3. Results

### 3.1. Drosophila tensin mutation causes obvious cellular abnormalities during oogenesis

To determine whether *Drosophila* tensin is involved in the regulation of dynamic cellular changes during oogenesis, we first examined the egg phenotypes of *by* mutants. In this study, we used two *by* mutant lines, P-element-inserted $by^2$ and $by^{ex49}$, one of the imprecise excision alleles of $by^2$ (Fig. S1A). According to a previous study, the gene expression levels of *by* in $by^2$ and $by^{ex49}$ were highly reduced and completely abolished, respectively [16]. To confirm this in the context of oogenesis, we conducted RNA *in situ* hybridization experiment with *Drosophila* ovaries. Consistent with the previously reported RT-PCR data, *by* expression levels in the ovaries of $by^2$ (Fig. S1C) were highly reduced compared to its abundant expression in the wild-type ovaries (Fig. S1B).

Notably, both homozygous mutant females for $by^2$ and $by^{ex49}$ and their *trans*-heterozygous mutant females laid significantly fewer eggs than wild-type flies, and the survival rate of the laid eggs also reduced in comparison to wild-type (Fig. 1A). However, the number of eggs laid by the revertant flies ($by^{rv8}$) that was generated by precise excision of inserted P-element in $by^2$ was similar to that of wild-type flies (Fig. 1A), further confirming that the female sterility in the *by* mutants is due to loss of the *Drosophila* tensin protein.

Interestingly, *by* mutant eggs exhibited a round-shape phenotype compared to wild-type eggs, and the analyses of the dimensions of the individual $by^2$ egg chamber showed that the mutant egg chamber failed to elongate properly during oogenesis (Fig. 1B and C). The length of late-stage egg chambers of $by^2$ appeared to be reduced about 25% compared to the wild-type control (Fig. 1C). In addition, homozygous females frequently contained degenerated and rudimentary ovarioles with no obvious septum between each egg chamber due to lack of inter-follicular stalk cells (Fig. 1D). We could observe even completely fused egg chambers in a few occasions. As shown in Fig. 1E and F, fused egg chambers of *by* mutant often contained upto twice more cells than the wild-type control. It was measured that two oocytes and about 30 nurse cells were found in a single fused egg chamber of *by* mutants. These data collectively suggest that *Drosophila* tensin may play a crucial role during oogenesis, which is further supported by our observation of high level of *by* expression in the ovariole particularly at the early germarium stage (Fig. S1B, arrow) when the follicle stem cells start to divide.

### 3.2. Drosophila tensin regulates border cell migration and polarity formation that both require proper organization of F-actin structures

We then decided to look into the cellular basis of aforementioned egg phenotypes of *by* mutants. Firstly, from the markedly decreased hatching rate of *by* mutant eggs (Fig. 1A), we suspected the possibility that *by* mutation may impair border cell migration which is an essential event for proper egg chamber development and egg fertility [2]. We monitored border cell migration by tracing FasIII-labeled anterior polar cells that are known to migrate together with border cells during *Drosophila* oogenesis [2]. While 90% of border cells from the wild-type egg chambers migrated

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