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Exocyst Regulates *Drosophila* Border Cell Migration and Wing Development

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Abstract. Cell migration plays an important role in many physiological and pathological processes. Understanding of the mechanisms of cell migration may lead us to develop novel therapeutic strategies for controlling human disease. Border cell migration in the ovary of *Drosophila melanogaster* has emerged as an excellent model for studying cell migration in *vivo*. In a previous study, we have found that the gene *sec3* that encodes a component of *Drosophila* exocyst is required for border cell migration. In the following study, we found that high levels of Sec3-GFP are seen in the leading edge of migratory border cells, and *sec3* functions together with other genes encoding exocyst components. Moreover, knockdown of exocyst components induces aberrant wing development. The results implicates that exocyst components play important roles in *Dosophila* border cell migration and wing development, and function as a complex as they have been found in yeast.

1. Introduction

The exocyst complex is conserved between species and comprises eight proteins: Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84.The exocyst complex was originally identified in the budding yeast *Saccharomyces cerevisiae* and shows to be essential for exocytosis. Without the function of exocyst, secretory vesicles can be delivered to sites of secretion, but they can not fuse with the target membrane(1). In animal cells, the exocyst seems to play a similar function in exocytosis.

The Drosophila ovary is composed of egg chambers. Each egg chamber contains 16 germline cells and about 650 somatic cells, called follicle cells. One of the germline cells differentiates into the oocyte, while the other 15 become nurse cells. In early oogenesis, a pair of special follicle cells forms at each end of the egg chamber, which called polar cells. At the beginning of stage nine of oogenesis, the anterior polar cells recruit a group of 4-8 cells from the adjacent follicular epithelium. These 6-10 cells formed border cell cluster. The border cells round up, detach from the follicle epithelial cells. Over a 6-hour period, they migrate between the nurse cells, forge their way through the centre of the egg chamber and reach the anterior border of the oocyte
at early stage ten of oogenesis, hence they get their name (2). In the last decates, border cell migration in the ovary of *Drosophila melanogaster* has emerged as a simple model for studying cell migration.

2. Sec3 Shows Polarized Localization in Egg Chamber.

We have identified loss of function of *Drosophila sec3* causes border cell migration defects in a previous study (3). Since the expression pattern of Sec3 in *Drosophila* has not been reported yet, we wanted to examine the protein localization of Sec3 and determine whether it shows a polarized localization in cells as other exocyst component having been reported. We generated *sec3-GFP* transgenic fly and expressed *sec3-GFP* by *Actin-Gal4*. In the germarium, high levels of Sec3-GFP protein were observed. Up to stage 6, Sec3-GFP is mainly localized close to the apical membrane of follicle cells (Figure 1A-A'). At stage 9 and 10, high levels of Sec3-GFP are seen in the leading edge of migratory border cells (Figure 1B-B'). In summary, we found a polarized localization pattern of Sec3-GFP in follicle cells and border cells.

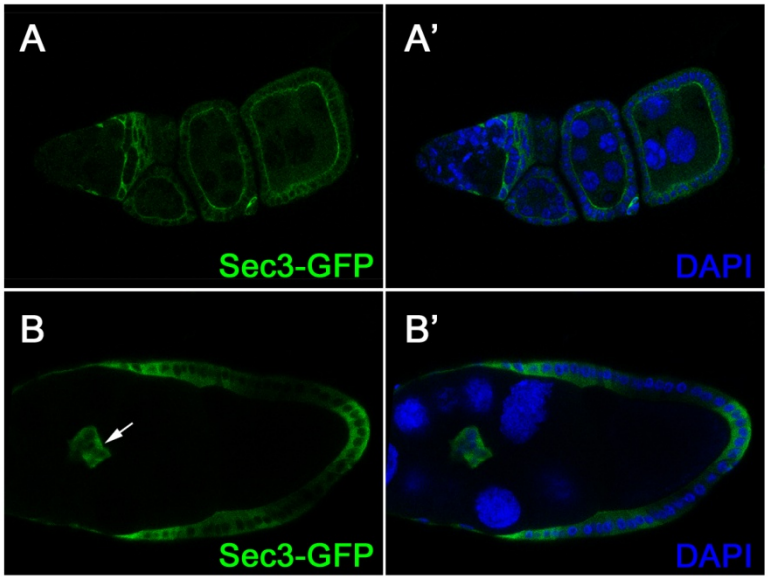

Fig. 1 Expression patterns of sec3-GFP in egg chamber. (A-D') Nuclei are labeled by DAPI (blue). (A-A') Up to stage 6, Sec3-GFP proteins are abundant at the apical side of follicle cells. (B-B') A stage 9 egg chamber, with high levels of Sec3-GFP protein at the leading edge of border cells (arrow)

3. Sec3 Works Together With Other Exocyst Components During Border Cell Migration.

Sec3 is exceptional in several respects of the eight exocyst proteins. Under some conditions, Sec3 is not necessary for growth and secretion in the budding yeast(4, 5). Sec3 was proposed to act as a spatial landmark for sites of polarized secretion, because Sec3-GFP fusion proteins localized to presumptive bud sites even in the condition of disruption of the function of other exocyst proteins(6). Sec3 was the only exocyst protein that was not delivered to the sites of secretion on transport vesicles(5). Although it is clear that the exocyst complex is required for tethering the secretory vesicle to the target membrane within the exocytic systems, it is not known whether the same exocyst holocomplex regulates each of the trafficking events. We had found that the Sec3 protein is required for border cell migration and it regulates several molecules essential for border cell migration. Then, we tried to figure out whether other exocyst proteins also involved in border cell migration, and whether mutants of other exocyst proteins show the same phenotypes as *sec3* mutant.

To find out whether Sec3 works together with other exocyst proteins in border cell migration, we did genetic interaction experiments. We specifically expressed different dsRNAs against the exocyst proteins in border cells by using *Slbo*-GAL4. Expression of RNAi can just partially knock down the target gene, and most of the expressions of RNAi does not affect border cell migration. But the combination of each RNAi expression with one copy of *sec3-d* causes border cell migration defect (Fig 2). These genetic interaction demonstrates that Sec3 works together with other exocyst proteins in border cell migration.

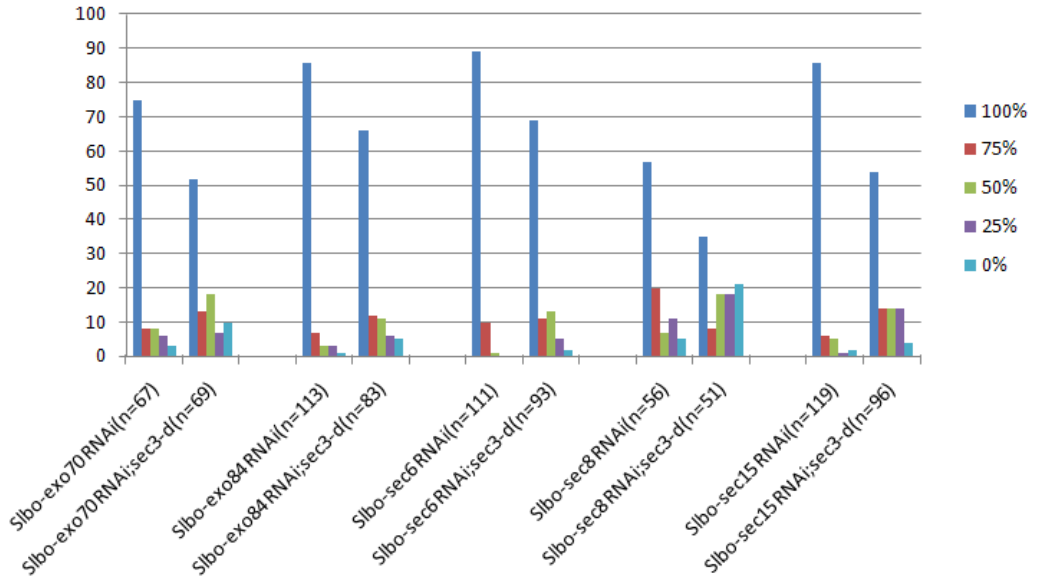

Fig. 2 Genetic interaction of Sec3 with other exocyst proteins. Except RNAi of sec8, expression of each RNAi drived by Slbo-GAL4 does not cause strong migration defect. In heterozygote of sec3-d (sec3 mutation), expression of each RNAi in border cells disrupts border cell migration. It indicates that loss of sec3 function can enhance the phenotypes of gene knocking down of other exocyst components.

4. Knockdown of Exocyst Components Results in Aberrant Wing Development

When we knocked down *sec3* with RNAi expressions driven by *MS1096-Gal4*, we observed striking effect on wing development and severe wing phenotypes (Figure 3B, C). As Sec3 is a component of exocyst, we knocked down genes of other exocyst components with RNAi expressions by *MS1096-Gal4*, and induced significant different wing phenotypes (Figure 3D). Therefore, the exocyst complex appears to have effects in wing development.

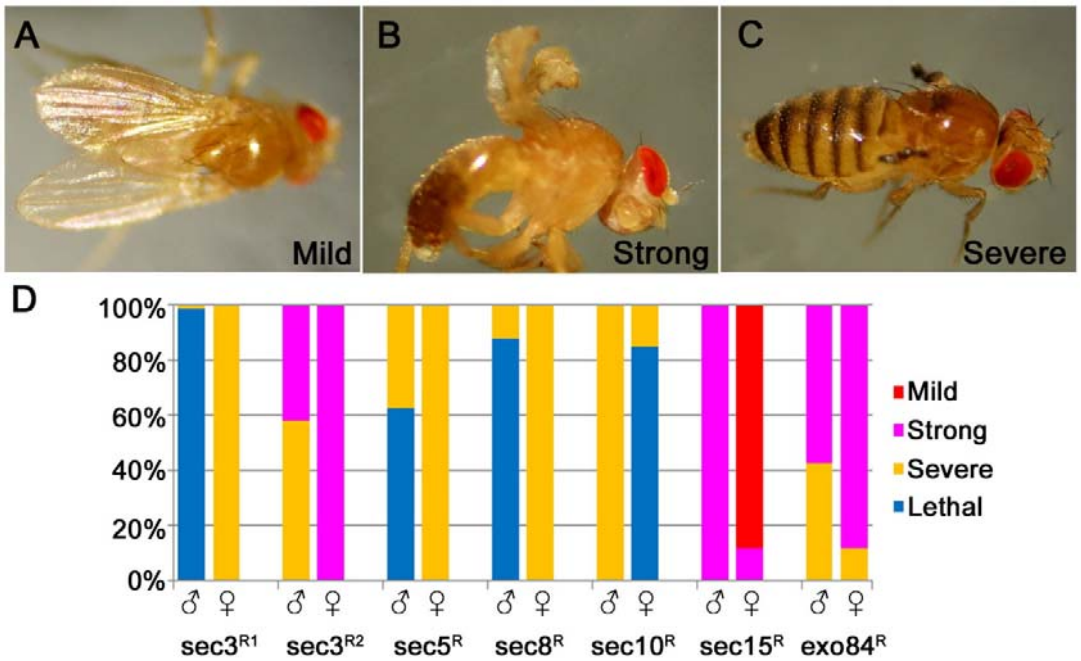

Fig.3 Components of exocyst are important for proper wing development. (A-C) Three examples showing mild wing defect (A), strong wing defect (B) or severe wing defect (C). (D) The F1 progeny of MS1096-Gal4 crosses each RNAi were scored for the wing defects degree (70<n<100). The number of lethal flies is got according to the 1:1 theoretical ratio of male progeny to female progeny.

Acknowledgements

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