Investigating the Function of the *shep* Gene in Cell Migration

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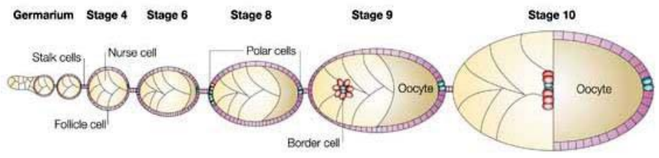
Abstract

Cell migration is an important process as it is necessary for proper immune function, embryonic development, and injury repair. Border cell migration in *Drosophila melanogaster* is used as a model to study cell migration due to similarities between fly genes and human genes, the ease of manipulating gene function in flies, and the fact that migrating cells can be observed in their native tissue. During border cell migration, a group of cells travels from one side of a developing egg chamber to the other. Previous investigations into a *D. melanogaster* gene called *shep* have indicated it may have a role in this important developmental process. We tested this hypothesis using immunohistochemistry, RNA interference, and a transposase experiment. A GFP reporter insertion in the *shep* locus showed specific expression of Shep protein in the border cells. Knockdown of *shep* via RNA interference caused delayed migration in developing eggs. The transposase experiment to generate *shep* alleles is ongoing but has not yet yielded affected offspring. Thus, another method to generate mutant alleles, CRISPR-Cas9, will be used. This work may reveal that Shep regulates cell migration in *D. melanogaster* and lead researchers to investigate the functions of similar proteins in humans.

Background

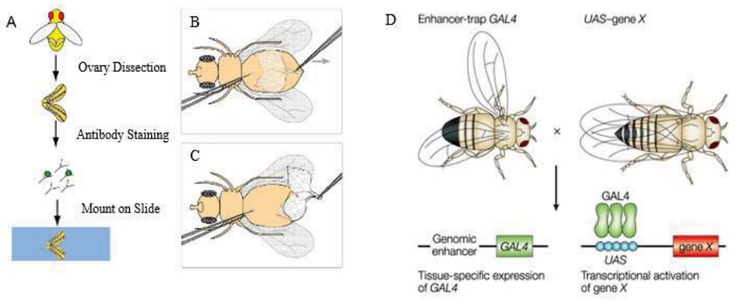


Montell, D. Border-cell migration: the race is on. Nat. Rev. Mol. Cell Biol. 2003; 4(1):13-24.

Figure 1. Border cell migration during *Drosophila* egg development. The ovariole of a *Drosophila* ovary contains egg chambers in different stages of development. In stage eight, the border cells differentiate from surrounding epithelial cells. They migrate in stage nine and arrive at the oocyte in stage ten.

Figure 2. *shep* genomic locus showing c522 and CC00236 insertion sites. The insertion in c522 flies has been found to have expression in the border cells. The area around the insertion was cloned and found to be in the *shep* gene. The c522 fly line contains an insertional mutant allele of the *shep* gene. Source: FlyBase

Methods



Wang, X, et al. Purification of specific cell populations from Drosophila tissues by magnetic bead sorting, for use in gene expression profiling. Protocol Exchange (2008).
St Johnston, D. The Art and Design of Genetic Screens: Drosophila melanogaster. Nat. Rev. 2002; 3: 176-188.

Figure 3. Ovary antibody staining and the GAL4-UAS system. A) *Drosophila* ovaries are removed under a microscope with forceps then stained using antibodies. B) The ovaries are located in the abdomen of the female *Drosophila*. C) The forceps pull the ovaries out of the abdomen. D) The Gal4 protein binds to the UAS, which activates transcription of GFP. The GAL4 gene is under the control of the c522 locus.

Methods

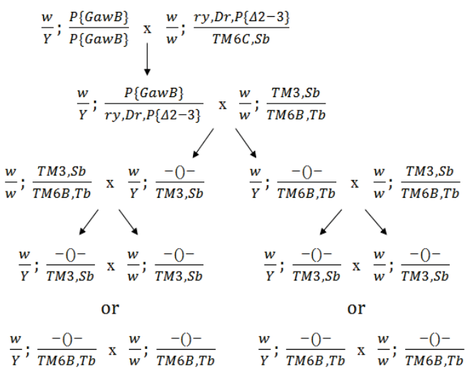


Figure 4. Strategy to generate new mutant alleles for the *shep* gene. Flies with a gene insertion for transposase are crossed with c522 flies. The transposase recognizes the P-element transposon in the c522 line and removes it. The imprecision of the transposase causes it to also remove part of the *shep* gene.

Results

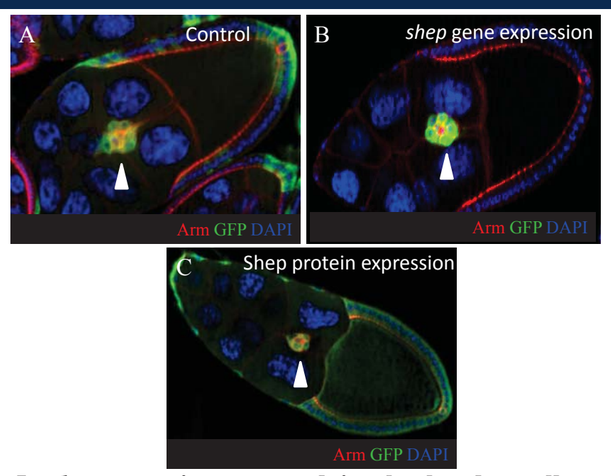


Figure 5. *shep* gene is expressed in the border cells and Shep protein is present in the border cells. A) Control slbo-Gal4/UAS-GFP egg chamber. B) Crossing c522 Gal 4 flies with UAS-GFP flies results in expression of GFP, marked in green. The P-element containing the Gal4 driver is inserted into the *shep* gene. This tells us *shep* is expressed in the border cells. C) CC00236 flies have the GFP gene spliced into *shep*. Thus GFP, marked in green, shows where Shep protein is located. We see here that it is located in the border cells and follicle cells. Nuclei are stained with DAPI (blue) and cell membranes are stained with anti-Armadillo (red). White arrowheads point to border cell clusters.

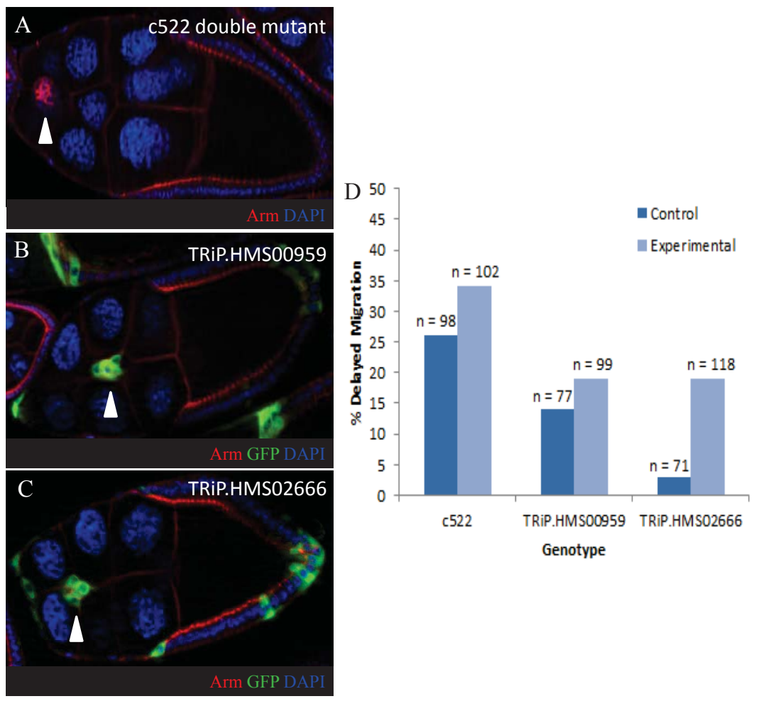


Figure 6. Disruption and knockdown of *shep* delay border cell migration. A) Stage 9 c522 double mutant with delayed migration. B) Stage 9 TRiP.HMS00959 (*shep* knockdown) with delayed migration. C) Stage 9 TRiP.HMS02666 (*shep* knockdown) with delayed migration. D) When the c522 insertion is on both chromosomes, the disruption causes migration delays in about 34% of egg chambers. Knockdown of *shep* with RNAi resulted in delayed migration in 19% of egg chambers.

Results

	P{GawB}	ry, Dr, P{Δ2-3}
TM3, Sb	133 $\frac{P\{GawB\}}{TM3,Sb}$	91 $\frac{ry,Dr,P\{\Delta2-3\}}{TM3,Sb}$
TM6B, Tb	168 $\frac{P\{GawB\}}{TM6B,Tb}$	128 $\frac{ry,Dr,P\{\Delta2-3\}}{TM6B,Tb}$

Figure 7. Transposase experiment F_2 offspring. Punnett square displays number of offspring with each genotype. The offspring of interest are those with ΔP{GawB} which should have white eyes.

Conclusions and Future Directions

- *shep* is expressed in the border cells.
- Shep protein is present in the border cells.
- *shep* disruption and knockdown delays border cell migration.
- F_2 offspring in transposase experiment indicate the insertion could be damaged, there is more than one insertion, or the insertion is moving around the genome.
- Taken together this evidence supports the hypothesis that *shep* does have a role in *Drosophila* border cell migration.
- Continue to gather and count F_2 offspring from transposase experiment.
- Use modern genetic techniques to create new mutant alleles by targeting and cutting *shep*.
- Dissect and stain mutant flies to analyse effects on border cell migration.

References

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