A Screen for Genetic Modifiers of Protein Phosphatase 1 Function in Drosophila Collective Cell Cohesion and Migration

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INTRODUCTION

- Collective cell migration is important in normal physiological processes, such as embryonic development, as well as abnormal processes such as cancer.
- *Drosophila melanogaster* border cells demonstrate developmentally regulated collective cell migration during oogenesis making it an excellent genetically accessible model for identifying how cell collectives move in tissues.
- During ovarian development, 6-8 cells form the border cell cluster, which migrate together as a cohesive cluster to reach the large oocyte at the posterior end of the egg chamber.

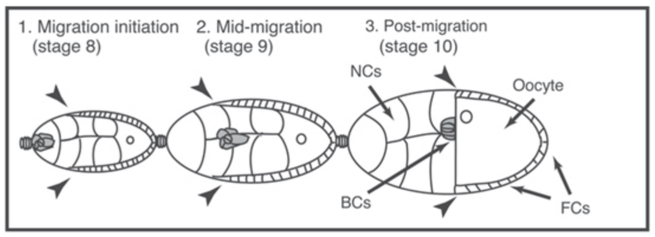
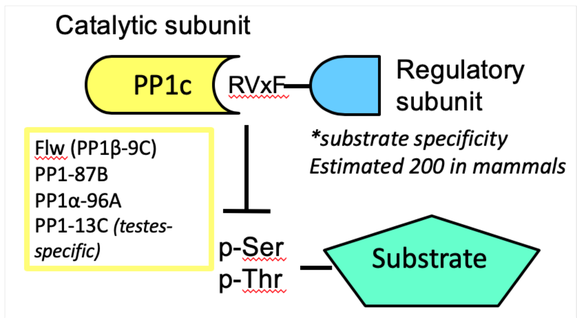


Figure 1: Ovariole egg chambers at developmental stages 8-10. NC, nurse cells; BC, border cells; FC, follicle cells. Arrowheads indicate rearrangement of follicle cells [1]

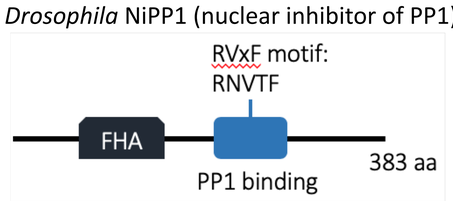
- Previous experiments from our lab have found that inhibition of protein phosphatase (PP1) activity, through overexpression of the endogenous (and specific) PP1 inhibitor, nuclear inhibitor of PP1 (NiPP1), caused the border cell cluster to separate into single cells and limited migration ability.
- Further experiments demonstrated that PP1 regulates actomyosin contractility and adhesion between border cells to promote collective migration.

Protein Phosphatase 1 (PP1)



NiPP1 inhibits PP1 activity

- *Drosophila* NiPP1 (nuclear inhibitor of PP1)



- NiPP1 is a:
  - PP-1 interacting protein
  - Endogenous protein inhibitor of PP1
  - Specifically inhibits PP1 activity in *Drosophila* and in vitro [2]
  - Potent inhibitor of PP1 activity in mammalian cells [3]

THESIS STATEMENT

By identifying genes that modify the NiPP1 phenotype, we will be able to determine PP1 molecular targets and pathway members.

METHODS

- To gain additional insights into how PP1 activity controls collective cell migration, we performed a genetic modifier screen of the NiPP1-induced border cell phenotypes.
- We screened the majority of deficiency lines from the 2nd and 3rd chromosome Bloomington Deficiency Kits, specifically looking for chromosomal regions whose altered gene dosage either enhanced or suppressed the effects of NiPP1 on border cell cohesion or migration.
- The GAL4/UAS system is used to express NiPP1.
- *slbo*-Gal4 (green, Figures 3 and 4) drives specific expression of UAS-NiPP1 in border cells, plus follicle cells.

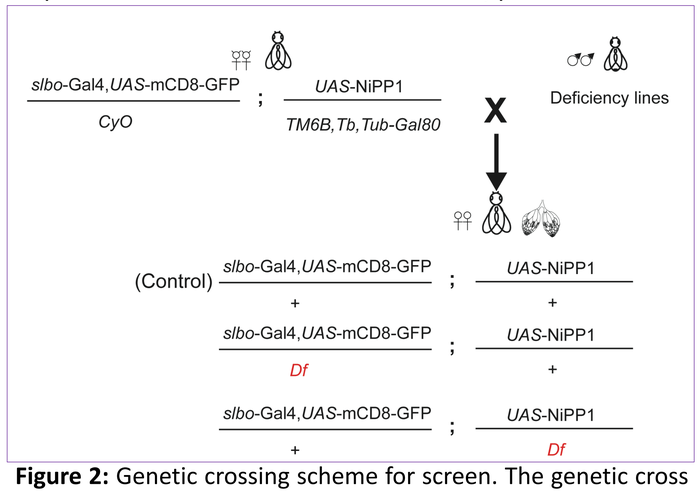


Figure 2: Genetic crossing scheme for screen. The genetic cross shows that progeny without CyO and TM6B (Tb+) will have the deficiency, *slbo*-GAL4, and UAS-NiPP1: these progeny are dissected and analyzed for migration and cohesion of the border cell cluster (see Figures 3 and 4).

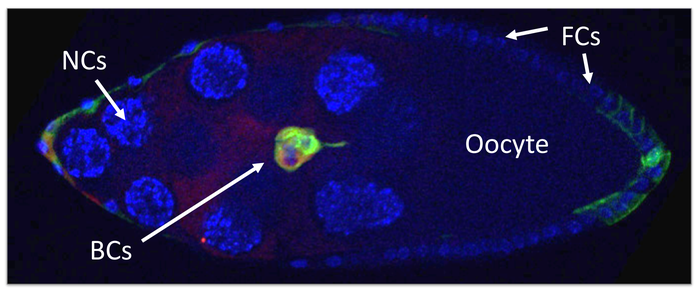


Figure 3: Normal border cell cluster and direction of migration at stage 9 oogenesis. Border (BCs), Nurse (NCs), Follicle Cells (FCs), and oocyte are labeled.

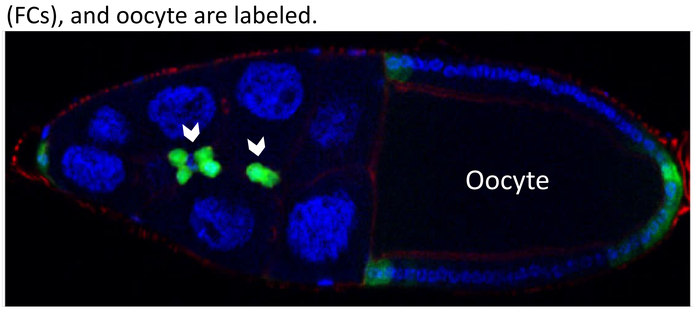


Figure 4: Adhesion and migration is disrupted when NiPP1 is over expressed; arrowheads indicate adhesion disruption and migration defects.

RESULTS

- We have now identified five distinct deficiencies that significantly enhance the NiPP1 migration defect and one deficiency that strongly enhances the NiPP1 cluster separation phenotypes.
- We are currently mapping the relevant genetic enhancers through a combination of testing smaller overlapping deficiencies and testing for interaction with specific RNAi lines.
- It is expected that the relevant smaller deficiencies will enhance the phenotype as well.

Summary of NiPP1 modifier screen using Bloomington deficiencies

Arm	Df lines tested (N)	Coverage (%)	Positive (N)	BDSC STOCK NUMBERS
2L	54	52	1	Df(2L)BSC209
2R	32	34	0	
3L	20	25	1	Df(3L)ED201
3R	18	17	3	Df(3R)ED5938, Df(3R)ED6096, Df(3R)slo3

Figure 5: Shows the number of deficiencies tested in the genetic screen of modifier NiPP1. About 86% of the 2nd chromosome (2L + 2R) has been tested with resulting one positive modifier. The 3rd chromosome coverage is 42% (3L + 3R) with four positive modifiers.

Positive Modifiers Migration Percentage at Stage 10

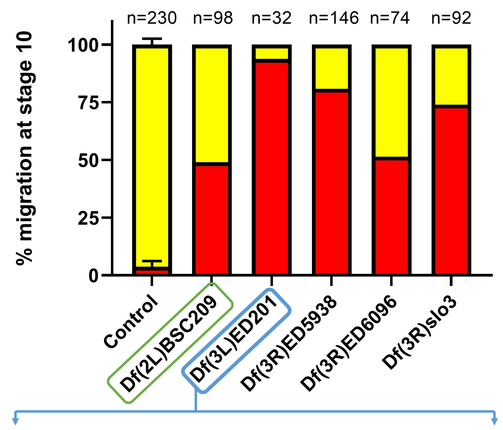


Figure 6: Shows positive modifiers compared to NiPP1 control. There is a significant increase in "no migration" of border cells suggesting phenotypic enhancement of the NiPP1 migration defect.

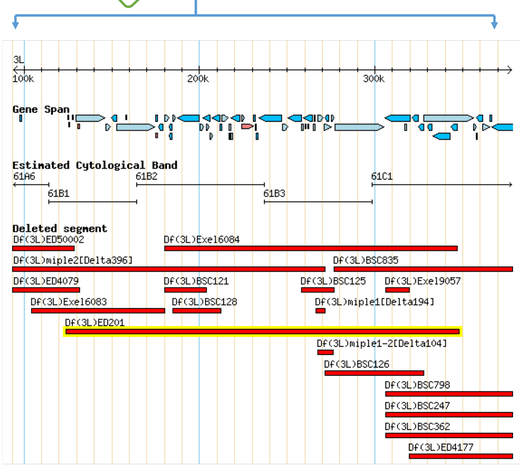


Figure 7: FlyBase image shows the deleted segment, Df(3L)ED201 highlighted in yellow, of chromosomal band; including associated small deficiencies.

- This deletion removes a single ribosomal protein coding gene (although other genes are also removed) [4].
- Six smaller deficiencies from Df(3L)ED201 are currently being analyzed.

CONCLUSION AND FUTURE RESEARCH

- By blocking PP1 activity in *Drosophila* border cells we were able to analyze deficiencies specifically to find which chromosomal segment enhanced or suppressed the NiPP1 phenotype such as migration defects, more "rounded" border cells, and weakened adhesion.
- So far in our genome-wide screen of the 2nd and 3rd chromosomes, we have found five positive modifiers of the NiPP1 migration defects.
- Smaller deficiencies from the positive modifiers are being analyzed and are expected to enhance the NiPP1 phenotype.
- After completing this analysis, RNA interference will be used to knockout specific genes and proteins to identify the PP1 molecular targets and pathway members.
- Identifying these targets and pathways members can be used to future study normal or abnormal processes in humans such as embryonic development and cancer.

REFERENCES AND ACKNOWLEDGEMENTS

[1] McDonald J.A., Montell D.J. (2005) Analysis of Cell Migration Using *Drosophila* as a Model System. In: Guan JL. (eds) Cell Migration. Methods in Molecular Biology, vol 294. Humana Press.

[2] Parker et al., *Biochem J.* 2002; Bennett et al., *Genetics* 2003.

[3] Winkler et al., *J. Cell Sci.* 2015

[4] Marygold et al., 2007.

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