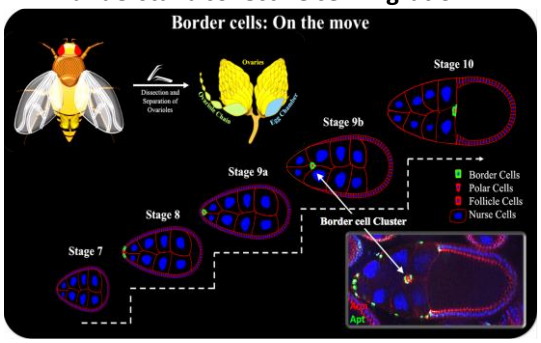
Mib2: A Key Regulator of Cytoskeleton and Border Cell Migration in *Drosophila* Egg Chambers

Sunny Trivedi, Michelle Starz-Gaiano, Biological Sciences, University Of Maryland Baltimore County, MD

ABSTRACT

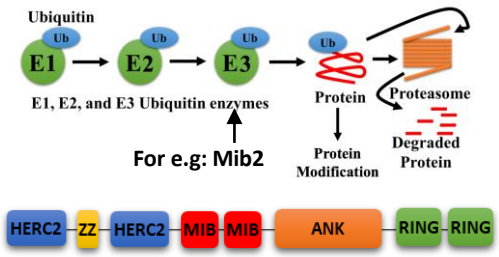
Collective cell migration is one of the many known biological mechanisms involved in organogenesis, and therefore is critically required. Border cells in *Drosophila* egg chambers are one of the most extensively used model to understand collective cell migration. JAK/STAT (Janus kinase/Signal transducer and activator of transcription) signaling is an extensively studied regulatory network for border cell migration (BCM) in *Drosophila* ovary that is also conserved and involved in organogenesis. In egg chambers, the anterior polar cells secrete Unpaired (Upd), which, when received by the surrounding follicle cells, activates the JAK/STAT cascade and specifies follicle cells into border cells[1]. Here, we propose to characterize Mind bomb 2 (Mib2) as a regulator of cytoskeleton proteins in fruit fly egg chambers. Mib2 is an E3 ubiquitin ligase involved in regulation of target protein fate. Mib2 is also important for the maintenance of Myosin in *Drosophila* musculature[3]. Earlier studies from our lab using RNA interference suggest the involvement of Mib2 in border cell migration[2]. We aim to characterize further the mode of action for Mib2 in migratory cell regulation and cellular cytoskeleton maintenance.

Figure 1
Using Drosophila egg chambers to understand collective cell migration



Border cells collectively migrate under tight regulation by many signaling pathways through nurse cells to reach oocyte in Drosophila egg chamber[1].

Figure 2
Mib2 is an E3 Ligase with conserved and well studied domain organization



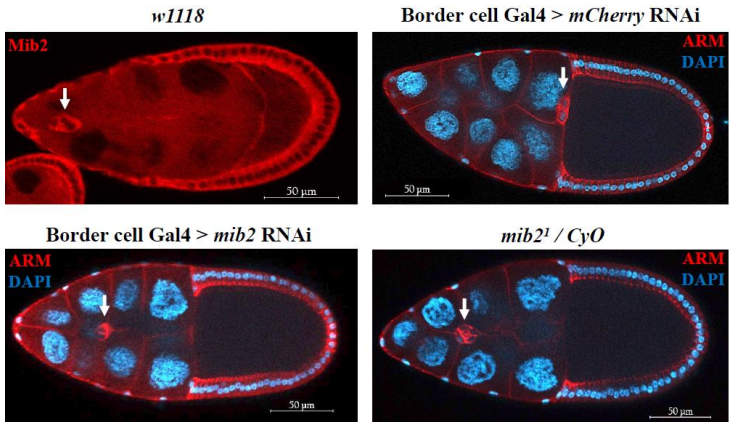
A) Ubiquitination pathway B) Mib2 domain organization (Adapted from Domsch et al. 2017)

Figure 3
mib2* is a negative regulator of STAT activity *in vitro

Gene name	dsRNA ID	z-score [6x2xDraf-luc]	z-score [4xSOCS-luc]	Functional group assignment (based on GO and Interpro evidence)
CG15706	HFA06577	2.2	2.1	Unknown
CG16975	HFA02552	2.7	2.7	Transcription regulators
CG17492 **Mib2**	HFA02623	2.5	2.1	Protein modifying enzymes / Metabolism
CG18112	HFA15304	2.1	2.1	Unknown
CG30122	HFA06935	3.3	2.8	Transcription regulators

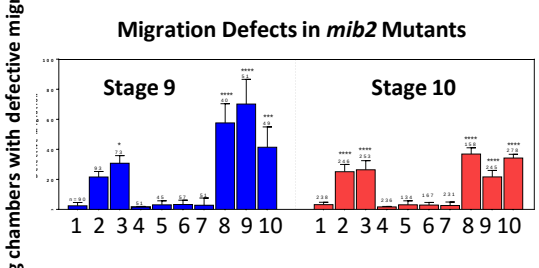
Jak/STAT signaling is required for the specification and migration of border cells
Muller et al. 2008, showed that Stat92E activity was upregulated in mib2 knockdown in vitro using Drosophila S2 cell line.

Figure 4
Mib2 is required for timely border cell migration



Mib2 is expressed in follicle cells and required for border cell migration. The mib2 knockdown by RNAi or heterozygous mutant causes incomplete border cell migration.

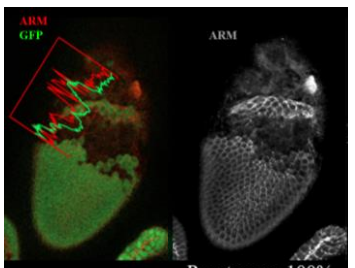
Figure 5
Specific domains of Mib2 seem to be important in the regulation of migration



1. w1118
2. *mib2* RNAi
3. *mib2*$^{(1)}$/Cyo
4. c306>UAS-*mib2*
5. c306>UAS-*mib2*$^{\Delta herc2a}$
6. c306>UAS-*mib2*$^{\Delta zz}$
7. c306>UAS-*mib2*$^{\Delta herc2b}$
8. c306>UAS-*mib2*$^{\Delta mib}$
9. c306>UAS-*mib2*$^{\Delta ank}$
10. c306>UAS-*mib2*$^{\Delta ring}$

Quantification of migration defects in different Mib2 mutants. The deletion lines of MIB, ANK and RF domains in wildtype background hinders migration efficacy. c306 drives expression in anterior follicle cells including border cells.

Figure 6
Mib2 may regulate β-catenin (Arm in flies)



The mib2¹ homozygous mutant clone patches are marked with absence of GFP. The mutant cells in the ovary seems to have reduced or un-organized Arm expression.

Besides Arm, we also see reduced expression of other cytoskeleton proteins in the *mib2*^{1} mutant clones. The data leads us to believe that Mib2 has a pleiotropic effect where it is required for the maintenance of cytoskeleton proteins which in turn are essential for cell migration.

In the future, we plan to investigate the role of other cytoskeleton proteins and involvement of Mib2 in Jak/STAT signaling

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REFERENCES: 1) Montell DJ, Yoon WH, Starz-Gaiano. Nat Rev Mol Cell Biol. 2012. 2) Saadin A, Starz-Gaiano M. G3 (Bethesda). 2016. 3) Marta Carrasco-Rando, Mar Ruiz-Gómez. Development 2008. 4) Müller P et al. Semin Cell Dev Biol. 2008. 5) Domsch K et al. PLoS One. 2017.