UNIVERSITY OF NEBRASKA AT OMAHA

FUNCTIONAL GENETICS OF *Mind-Meld* IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Immunohistochemistry (IHC) is a useful research tool used to localize specific antigens in tissue sections with labeled antibodies based on antigen-antibody interactions. To obtain a clearer understanding of the cellular and subcellular localization of the Mind-Meld (MMD) protein during the developing *Drosophila melanogaster* embryo double indirect immunofluorescence was used to colocalize MMD with other proteins found in the fruit fly. An affinity-purified antibody raised in rabbit identifying all MMD isoforms was used with the fluorophore-labeled secondary antibody Alexa Fluor 594 specific to rabbit IgG. Well-characterized murine monoclonal antibodies with known subcellular localization and function in *Drosophila:* Fascicilin II (FASII), Short stop (SHOT), β-Tubulin, Spaghetti squash (SQH), and Neurotactin (NRT) were used with the fluorophore-labeled secondary antibody Alexa Fluor 488 specific to mouse IgG. All fixed embryos were stained with the nuclear marker DAPI to identify the density and arrangement of the nuclei and the F-actin marker Phalloidin. Confocal microscopy which provides three-dimensional optical resolution was used to visualize the localization of the proteins. In the present work, it is demonstrated that MMD colocalizes with SHOT, β-Tubulin, Phalloidin and SQH suggesting MMD's importance in cell adhesion, cell migration and establishing the cytoskeletal. The characterization of the cellular and subcellular localization of the MMD protein during the developing *Drosophila* will provide insight into the context in which *mmd* functions in human disease processes.

INTRODUCTION

In multicellular organisms, intercellular signaling and adhesion are essential functions of every cell. In the nervous system, signals between neurons, as well as, neurons and glial cells play a pivotal role in nervous system homeostasis, and synapse function (Araque, & Navarrete 2010). The ADAMs (a disintegrin and metalloproteinase) are a family of transmembrane and secreted proteins that play an important role in regulating cell phenotype via cell adhesion, migration, proteolysis and signaling (Przemyslaw et al. 2013). Several ADAMs function in spermatogenesis, specifically in the maturation of sperm and their adhesion and migration in the uterus (Ikawa et al. 2010) while others function in the nervous system as guidance mechanisms and when disrupted are linked to cancer, cardiovascular disease, asthma and Alzheimer's disease (Liu et al 2009). Knowledge of protein localization often plays a critical role in characterizing the cellular function of hypothetical and newly discovered proteins. The *mmd* gene in flies is homologous to a member of the ADAM family, specifically the *ADAM23* gene. ADAM23 is predominantly expressed in the brain, suggesting that it may mediate cell adhesion through interactions with specific integrins (Cal 2000). Members of this family are structurally related to the typical structure of the ADAM family members; however, the metalloproteinase domain of ADAM23 suggests that its more significant role is in cell-cell and cell-matrix interactions, including fertilization, muscle development and neurogenesis (Klein & Bischoff 2011; Hu et al 2011). The characterization of the subcellular localization of the MMD protein during the embryogenesis of *Drosophila* against other proteins with well-known localization and function will provide insight into how *mmd* and its human homolog *ADAM2*3 function in human disease processes and further provide clues to understanding the mechanisms of human diseases. This research will use immunofluorescence and confocal microscopy to map and characterize a neuronally expressed ADAM protein, Mind-Meld (MMD) with the specific subcellular markers: Fascicilin II (FASII), Short stop (SHOT), β-Tubulin, Spaghetti squash (SQH), Phalloidin and Neurotactin (NRT) found in the fruit fly *Drosophila* melanogaster, to better understand its cellular function.

MATERIAL/METHOD

Primary antibodies (Developmental Studies Hybridoma Bank, Iowa City, IA): mouse ∝-Shot, mouse ∝-Spaghetti squash, mouse ∝-Neurotactin; mouse ∝- β-Tubulin; mouse ∝-Fascicilin II; rabbit ∝- MMD (B. Chase). Secondary Antibodies (ThermoFisher Scientific): goat ∝-mouse, Alexa Fluor Plus 488; donkey ∝-rabbit, Alexa 594; Phalloidin, Alexa 647.

Fixation and de-vitellinization

- Embryos were de-chorionated in 50% bleach, washed with 1% Triton X-100 and transferred to a 20 ml vial containing fixative (4 ml heptane, 3 ml phosphate-buffered saline (PBS), 1 ml 16% formaldehyde (Ultrapure)) and shaken for 25 min. The lower phase was removed and embryos were de-vitellinized by vigorous shaking in 4 ml cold MeOH for 1 min, transferred to a 1.5 ml (Eppendorf) tube, rinsed 3x with MeOH and stored in MeOH at -20℃.

Prewash

- Embryos were rinsed 2x with PBT (PBS, 0.1% Tween 20, 0.3% Triton X-100), washed by rocking for 4 x 15 min in 1 ml PBT, rocked for 1 hr in 1 ml PBTi (PBT, 250 mM imidazole), rocked for 2 hr in 1 ml hybridization buffer, rinsed in 1 ml PBT (2x) and rocked in 1 ml PBT for 15 min

Staining

- Embryos were incubated for 1 hr in 1 ml blocking solution followed by incubation for 14+ hr at 4℃ in 0.5 ml blocking solution + first primary antibody, rinsed by rocking at room temperature in 1 ml PBT for 30 min (10x), and blocked for 1 hr in 1 ml blocking solution. Embryos were incubated with secondary antibody in 0.5 ml blocking solution for 1.5 hr at room temperature, and rinsed in 1 ml PBT (2x). Staining was repeated with the second primary antibody and its corresponding secondary antibody. Embryos were stained with DAPI and Phalloidin in 1 ml PBT (5 min), washed in 1 ml PBT (4x) at 4℃ for 2–14 hr, and mounted in Vectashield on a back glass-bottomed dish.

RESEARCH

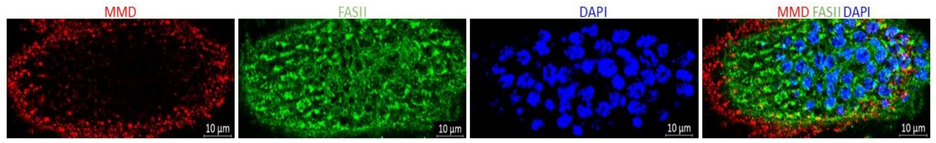

Figure 1. Expression of Mind-Meld protein and Fascicilin II protein during germ band elongation. A stage 10 embryo stained with antibodies against MMD, FASII, and the nuclear marker DAPI. The merged image is shown. 63X magnification

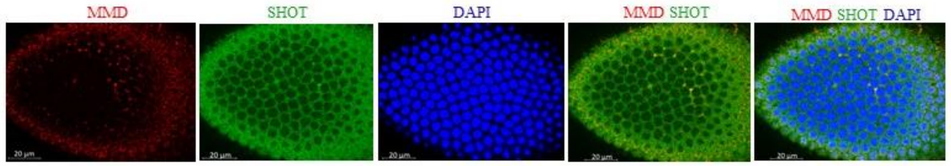

Figure 2. Expression of Mind-Meld protein and Short stop protein during cellularization. A stage 5 embryo stained with antibodies against MMD, SHOT and the nuclear marker DAPI. The merged images are shown. 40X magnification

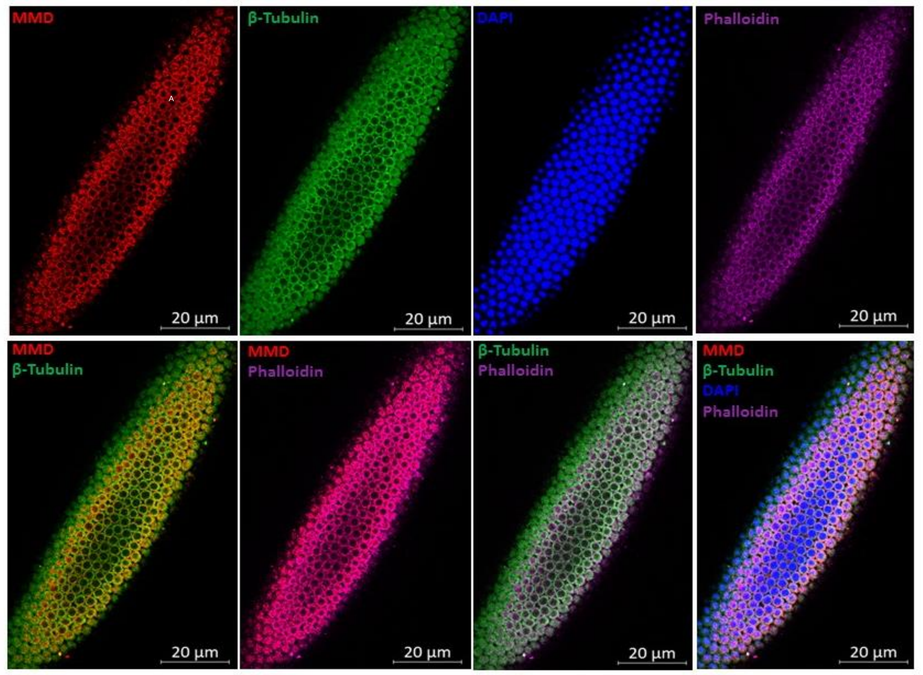

Figure 3. Expression of Mind-Meld protein and β-Tubulin protein; Mind-Meld protein and Phalloidin during blastoderm cell formation. A stage 3 embryo stained with antibodies against MMD, β-Tubulin, Phalloidin and the nuclear marker DAPI. The merged images are shown. 40X magnification

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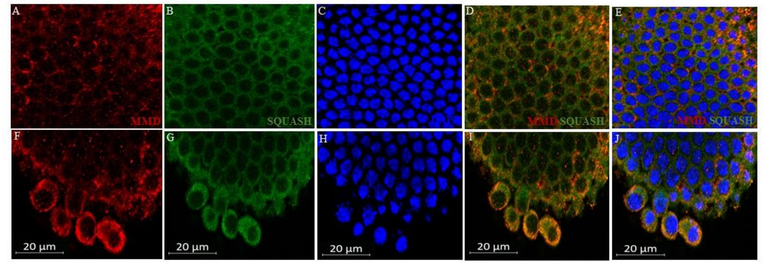

Figure 4. Expression of Spaghetti squash protein and Mind-Meld protein during shifting of the pole cells. A stage 5 embryo stained with antibodies against MMD, SQH and the nuclear marker DAPI. The merged images are shown. 40X magnification

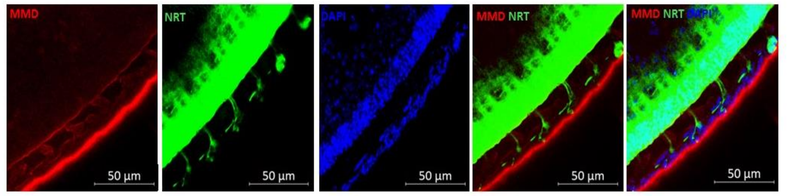

Figure 5. Expression of Neurotactin protein and Mind-Meld protein during neurogenesis. A stage 9 embryo stained with antibodies against MMD,NRT, and the nuclear marker DAPI. The merged images are shown. 20X magnification

CONCLUSION

- Although FASII is a cell membrane glycoprotein belonging to the immunoglobin superfamily of cell adhesion molecules, no colocalization was observed between FASII and MMD. Perhaps MMD is not involved in synapse development and plasticity.
- MMD and SHOT colocalized at the periphery during stage 5 of embryogenesis when cellularization is occurring with the elongation of the blastoderm nuclei. SHOT is important for establishing the cytoskeletal arrangement required for the axis determining maternal mRNAs. Therefore, the colocalization of SHOT and MMD at the outer periphery of the embryo suggests that MMD could play a role in the organization of microtubule that leads to the development of the oocyte polarity.
- MMD colocalizes with β-Tubulin during stage 3 of embryogenesis suggesting MMDs role in preposition of developmental cues in the oocyte and cytoplasmic reorganization in the embryo.
- MMD colocalizes with Phalloidin with high expression during stage 3 of embryogenesis. Phalloidin labels F-actin which is important for cell motility and phagocytosis. At the beginning of cellularization, MMD and F-actin form hexagons around the nuclei suggesting their role in forming a network to act as a contractile ring to cleave the fully formed cells from the underlying yolk sac.
- MMD colocalizes with SQH during stage 5 of embryogenesis especially surrounding the nuclei of pole cells. The *sqh* gene encodes the regulatory myosin light chain of non-muscle myosin II that is responsible for follicle cell migration. This suggests that MMD plays a role in the migration of pole cells to the dorsal end of the embryo.
- MMD does no colocalize with NRT. However, during stage 9 with the first neuroblasts delaminating from the ectoderm, MMD appears to also form a bridge like structure between regions of contact for neuroblasts.

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