MATERIALS/METHOD

Primary antibodies (Developmental Hybridoma Bank, Iowa City, IA): mouse n-Short, mouse n-Spaghetti squash, mouse n-Neurotactin; mouse g-Tubulin; mouse n-Fasciclin II; rabbit n-Mind-β (S. Chase), Secondary Antibodies (Vector Laboratories): goat anti-rabbit, mouse, Alexa Fluor 488, donkey n-rabbit, Alexa Fluor 594, Phalloidin, Alexa 647.

Fixation and de-vitellination

Embryos were de-vitellinated in 50% bleach, washed with 1% Triton X-100 and transferred to a 20 ml vial containing 6 volumes 4% formaldehyde, 3 ml phosphate-buffered saline (PBS), 1 ml 16% formalin (Ultrease) and shaken for 25 min. The lower phase was removed and embryos were de-vitellinated by vigorous shaking in 6 ml cold M25 buffer for 1 min, transferred to a 1.5 ml (Eppendorf) tube, rinsed 3x with M25 buffer and stored in M25 at -20℃.

Prewashing

Embryos were rinsed 2x with PBS (PBS: 0.1% Tween 2.0, 0.3% Triton X-100), washed by rocking for 4 x 15 min in 1 ml PBS, replaced for 1 hr in 1 ml PBS (200 μg/ml nucleases) rinsed in PBS 3x and transferred to 1 ml PBS. 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 PBS for 40 min (2x), and blocked for 1 hr in 1 ml blocking solution. Embryos were incubated with secondary antibody in 0.5 ml blocking solution for 1 hr at room temperature, and rinsed in 1 ml PBS (2x). Staining was repeated with the second primary antibody and then secondary antibody and embryos were stained with DAPI and Phalloidin in 1 ml PBS (50 μM), washed in 1 ml PBS (4x) at 4℃ for 2-14 hr, and mounted in Vectashield on a back glass dish.

ACKNOWLEDGEMENTS

• Dr. Bruce Chase, Professor, Department of Biology, University of Nebraska Omaha
• University of Nebraska Omaha Biological Sciences Research Funding
• University of Nebraska Medical Center Advanced Core Facility

REFERENCES


• Ikeda, M., Ora, N., Ishibashi, A., & Osada, H. (2013). Regulation of a transient migration of photoreceptor precursors is required for the axis determining maternal mRNAs. Therefore, the colocalization of SHOT and MMD at the outer periphery of the embryos suggests that MMD could play a role in the organization of microtubule that leads to the development of the corteclar polarity.

• MMD colocalizes with Phalin during stage 3 of embryogenesis suggesting MMDs role in postembryonic development cell cycles in the cytoplasmic reorganization in the embryo.

• MMD colocalizes with Phalin with high expression during stage 3 of embryogenesis. The phalin gene encodes the regulatory myosin light chain of non-muscle myosin II that is responsible for filar cell migration. This suggests that MMD plays a role in the migration of pole cells to the dorsal end of the embryo.

• MMD does not co-localize with NRT. However, during stage 9 with the first neuroblasts delaminating from the ecdysium, MMD appears to also form a bridge-like structure between regions of contact for neuroblasts.

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 cellulization 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 embryos suggests that MMD could play a role in the organization of microtubule that leads to the development of the corteclar polarity.

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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, & Navarro, 2010). The ADAMs (a disintegrin and metasplrotease) are a family of transmembrane and secreted proteins that play an important role in regulating cell phenotype via cell adhesion, migration, proteolysis and signaling (Prymala et al., 2015). Several ADAMs functions in spermatogenesis, specifically in the maturation of sperm and their adhesion and migration in the uterus (Ikeda et al., 2010) while others function in the nervous system as guidance mechanisms and when disrupted are linked to cancer, cardiovascular disease, autism and Alzheimer’s disease (Li et al., 2009). Knowledge of protein localization often plays a critical role in characterizing the cellular function of hypothetically and newly discovered proteins. The metagene 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 metagene 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 localized localization and function will provide insight into how mmd in human homolog ADAM23 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 recently expressed ADAM protein, Mind-Meld (MMD, Mind-β) with the specific subcellular markers: Fasciclin II (FASII), Short stop (SHOT), β-Tubulin, Spaghetti squash (SQH), Phalloidin, and Neurotactin (NRT) found in the fruit by Drosophila melanogaster, to better understand its cellular function.

FUNCTIONAL GENETICS OF Mind-Meld IN DROSOPHILA MELANOGASTER

Candi Senior-Remsa

Department of Biology, University of Nebraska at Omaha, NE 68122

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 fly. An affinity-purified antibody was used in initial identifying all MMD isoforms was used with the fluorescein-labeled secondary antibody Alexa Fluor 594 specific to rabbit IgG. Well-characterized murine monoclonal antibodies with known subcellular localization and function in Drosophila, Fasciclin II (FASII), Short stop (SHOT), β-Tubulin, Spaghetti squash (SQH), and Neurotactin (NRT) were used with the fluorescein-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 nucleus and the F-actin marker Phalloidin. Confocal microscopy which provides three-dimensional optical section was used to aid in 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 cytoskeleton. The characterization of the cellular and subcellular localization of the MMD protein during the developing Drosophila will provide insight into the context in which mind proteins function in human disease processes.

RESEARCH

In vitro imaging which provides three dimensional optical section was used to aid in 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 cytoskeleton. The characterization of the cellular and subcellular localization of the MMD protein during the developing Drosophila will provide insight into the context in which mind proteins function in human disease processes.

Figure 1. Expression of MMD-Meld protein and Fasciclin 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 MMD-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 MMD-Meld protein and β-Tubulin protein; Meld-Mind protein and Phalin 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

Figure 4. Expression of Spaghetti squash protein and Mind-Meld protein during shifting of the pole cells. A stage 9 embryo stained with antibodies against MMD, SHOT 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