The fine structure of the tentacular apparatus of *Mnemiopsis leidyi*

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Ctenophores are gelatinous zooplankton found throughout Earth's oceans, from the depths of oceanic trenches to the surface of shallow estuarine systems. Their unique morphology and physiology have long intrigued researchers working on various topics in development, neurophysiology, and evolution. Ctenophores have become popular research organisms because they were recently found to be the last common ancestor to all animals. I have investigated the cellular architecture of the ctenophore tentacular bulb and associated tentacles. The ctenophore Mnemiopsis leidyi was chosen because of its year-round availability and its resilient physiology. Mnemiopsis bears tentacles that arise from two centrally positioned tentacular bulbs. They have fine tentillae that extend perpendicular to the tentacles, which contain 'sticky' colloblast cells that aid in prey capture.

My goal was to visualize tentillar organization at a cellular level, using light microscopy and transmission electron microscopy (TEM). Preparations for microscopy were chemically fixed, infiltrated and embedded in Spurr's epoxy resin. I generated 0.25-micron thick cross sections of the tentacle bulb using an ultramicrotome, then stained and viewed at up to 100x under a microscope equipped with a digital color camera. Since slide preparation causes dehydration and collapse of the watery tissue, I additionally cryosectioned frozen ctenophore bulbs to determine if dehydration changed the structural profile. I also performed analysis of 60 nm thick 'thin sections' using TEM. I sectioned resin-embedded samples with glass knives, followed by staining with lead citrate and uranyl acetate. Thin sections were mounted on copper grids, dried and examined by TEM on the Zeiss EM10 microscope in Auburn University Research Instrumentation Facility (AURIF) at up to 30,000X magnification. We were able to form a high-resolution 'mega-image' (totaling 1.2 terabytes) of a tentacular bulb, revealing previously unseen and unknown cellular structures and cell-cell interactions.

We discovered the following: (1) the oral-most end of the bulb and canal was not attached to the body; (2) what we interpret to be a layer of food-absorptive cells that corroborates a recent report (Giribet, 2016); (3) a structural feature we interpret to be bulb neuropile surrounding a region rich in what we interpret to be neural stem cells; (4) a region of tentillar longitudinal fission and growth (TLFG) that 'waves' laterally to either side of the bulb to 'feed' tentillar structure within the tentacular groove of the food groove; and (5) several unknown cell types, including apparently apoptotic cells, at different stages of differentiation.

We view our findings as groundwork to investigate the function and identity of neuronal stem cells. These cells are of much interest because Mnemiopsis demonstrates the ability to rapidly regenerate and heal its wounds. Little is known about the function of the cells involved in tissue repair and how these cells interact with surrounding effectors. EdU (5-ethynyl-2'-deoxyuridin) analysis (Salic and Mitchison, 2008) by others showed a source of cell proliferation in the aboral portion of the ctenophore Pleurobrachia pileus (Alie et al., 2010). However, Pleurobrachia is structurally distinct from Mnemiopsis. Future studies will precisely determine the nature of the cells uncovered and relate them to their differential gene expression during cellular development.

References:

Giribet, G. 2016. Zoology: At last an exit for ctenophores. Curr. Biol. 26: R913–R936.
Alie, A. et al. 2010. Somatic stem cells express Piwi and Vasa genes in an adult ctenophore: Ancient association of "germline genes" with stemness. Dev. Biol. 350: 183.
Salic, A. and Mitchison, T. 2008. A chemical method for fast and sensitive detection of DNA synthesis in vivo. PNAS 105, (7):2415-2420.

Statement of Research Advisor:

Dorothy's work on the ultrastructural organization of the Mnemiopsis tentacular bulb and tentacular/tentillar structure has produced major strides in our understanding of this sensory integration center. This is a very difficult and time consuming, even tedious effort, and yet Dorothy's enthusiasm and boundless energy, and attention to detail, together with the careful overview of her graduate student supervisor in addition to myself, has broken open the cellular organization of this mysterious system. This work is of considerable importance because ctenophores are thought to be among the earliest metazoan (i.e., multicellular animals) to evolve, and are thought to therefore have one of the most ancient nervous systems. That the ctenophores are so ancient poses an interesting possibility: namely that they could be a potential precursor to all current nervous systems in more complex, 'higher' animals, including ourselves. —Anthony Moss, Biological Sciences