doi:10.1017/S1431927613002122
Microsc. Microanal. 19 (Suppl 2), 2013

Germ Granule Ultrastructure and Germ Line Protein Localization during Early Embryogenesis in Penaeid Shrimp

Kailey A. Vincent, Kristen M. Karasiewicz and Philip L. Hertzler

Central Michigan University, Biology Department, Mount Pleasant, Michigan, USA

Aquaculture is growing in importance to agriculture and provides close to 50% of the world's seafood consumption. As the global population increases the demand for improved and more sustainable aquaculture techniques become necessary. The global shrimp farming industry addresses the core of this increased demand [1]. To increase productivity, brood stock farms in the aquaculture industry have bred genetically superior lines of penaeid shrimp, and are very interested in methods to sterilize the postlarvae provided to farmers to protect their financial investment. Efforts to produce sterile shrimp by ionizing radiation and polyploidization have proven unsuccessful. An alternative approach to induce sterility would be to inhibit germ line formation by repressing the expression of germ line genes. Despite the huge economic importance of penaeid shrimp, little is known about the development of the germ line in this group of organisms.

The germ line is typically set aside from the somatic cells during embryogenesis and later gives rise to the gametes. Germ line formation occurs differently amongst organisms; however, the precursor cells of the germ line, the primordial germ cells (PGCs), eventually divide and migrate to the developing gonads where they will differentiate into germ line stem cells [2].

In many organisms the germ line is morphologically distinguishable from the somatic cells by the presence of distinctive granules, termed germ granules. In general, germ granule refers to the RNA-rich, electron dense, cytoplasmic bodies in germ cells. From the use of electron microscopy these granules have been observed to be non-membrane bound, compact aggregates that are believed to contain proteins and RNAs required for germ cell development [3].

A hypothesized germ granule has been identified in penaeid shrimp, termed the intracellular body (ICB). The ICB is localized to a single cell from the 4-cell stage to the 122-cell stage by fluorescent staining with Sytox Green or STYO RNASelect [4] and by TEM [5]. The ICB contains RNA and is composed of electron-dense granules, small vesicles, and multi-vesicular bodies. We have identified dense, granular material located in one of the two mesendoderm cells that may derive from the ICB in later stage embryos by TEM (Fig. 1). The fate of material derived from the ICB is currently being traced by TEM from the developmental stages beyond the 122-cell stage to the formation of the hypothesized PGC.

To confirm the identity of the PGC, antibodies to the known germ line proteins Vasa and Nanos were tested on penaeid shrimp embryos. Vasa and Nanos antibodies made to other organisms were not reactive in shrimp as determined by immunostaining and immunoblotting, so custom monoclonal antibodies were synthesized based on the shrimp Vasa and Nanos protein sequences. The results will be reported [6].

References:

[1] M Sellars *et al*, Aquaculture **272** (2007), pp. S309-S310.
[2] C Extavour, Developmental Biology **277** (2005), pp. 387-402.
[3] E Voronina *et al,* Cold Spring Harbor Perspectives in Biology (2011), 10.1101/cshperspect.a002774.
[4] JB Pawlak *et al*, Development, Growth & Differentiation **52** (2010), pp. 677-692.
[5] RM Grattan *et al*, Arthropod Structure & Development **In Press** (2013).
[6] This research was undertaken within the CSIRO Food Futures Flagship Cluster on "Sex ratio and sterility for commercial animal production" with funding from the CSIRO Flagship Collaboration Fund and Central Michigan University.

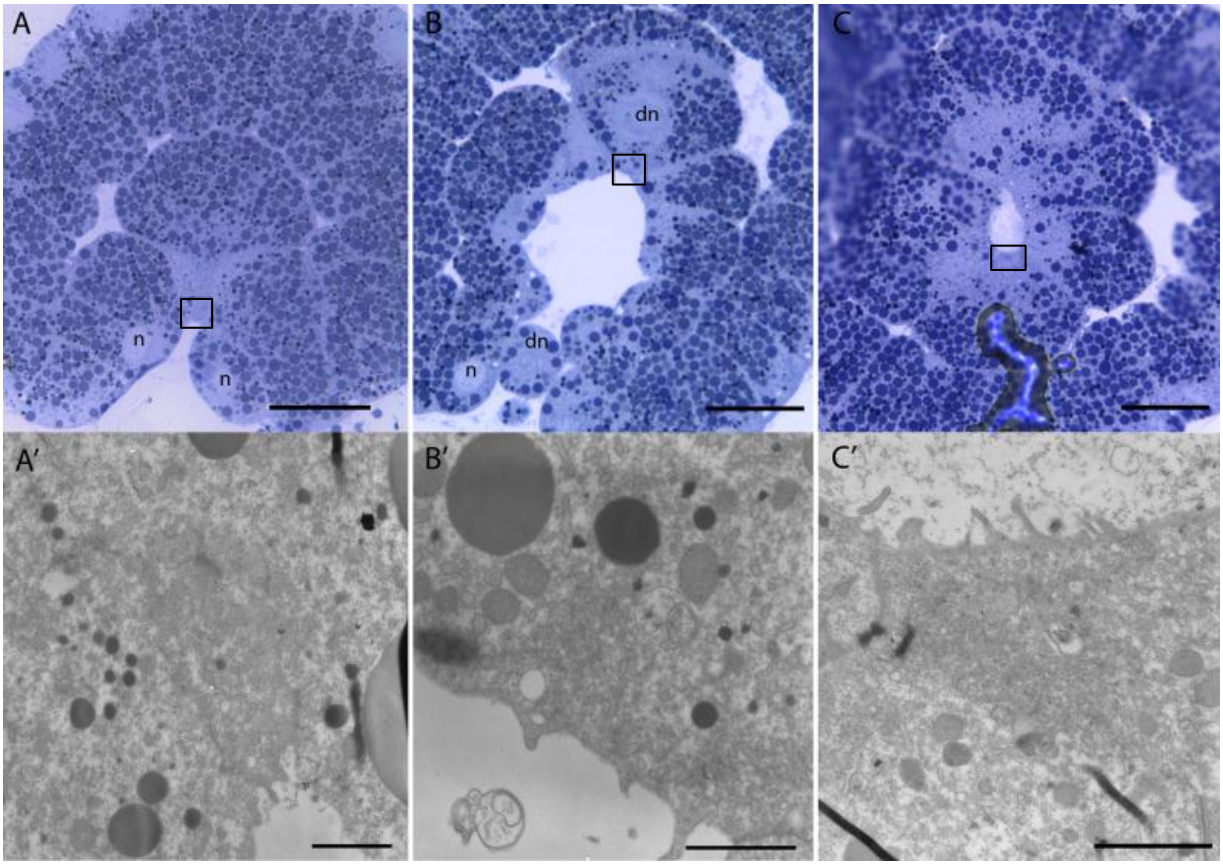

Figure 1. Three embryos of *Marsupenaeus japonicus* at 3 hrs (A), 3.25 hrs (B), and 3.75 (C) hrs post-fertilization. Top row: toluidine blue stained thick sections of developing embryos. Box indicates location of putative ICB. Bottom row: ultra-thin sections imaged using TEM displaying granular tentative ICBs. Scale bars: A, B, and C = 30 μm, A' = 10 μm, B' = 5 μm, C' = 3 μm. Abbreviations: n= nucleus, dn = degrading nucleus.

https://doi.org/10.1017/S1431927613002122 Published online by Cambridge University Press