2011

Characterization and three-dimensional organization of nuclei within developing spermatogenic cysts in Drosophila pseudoobscura

Crystal Pristell
Seton Hall University, Crystal.Pristell@shu.edu

Angela V. Klaus
Seton Hall University, Angela.Klaus@shu.edu

Follow this and additional works at: http://scholarship.shu.edu/bio-student-work

Part of the Biology Commons

Recommended Citation
http://scholarship.shu.edu/bio-student-work/1
Characterization and three-dimensional organization of nuclei within developing spermatogenic cysts in *Drosophila pseudoobscura*

Crystal Pristell and Angela V. Klaus

Department of Biological Sciences, Seton Hall University, South Orange, NJ, USA

**BACKGROUND**

Previous work in our laboratory was aimed at the development of an in vitro system for culturing *Drosophila* sperm cells. The current work is aimed at analyzing spermatogenic cyst morphology so that we can accurately characterize cyst maturation in our in vitro culture system. Sperm precursor cells develop within cysts and eventually mature to produce motile, elongate sperm cells. Germine stem cells are maintained in the stem cell niche in the apical end of the testis. Germine stem cells differentiate and become encapsulated in a cyst. After encapsulation, the germline cell (called a "gonialblast") at this stage) undergoes series of divisions which increase the number of sperm precursors within the cyst. In *D. pseudoobscura*, there are five mitotic divisions, followed by two meiotic divisions, resulting in 124 haploid cells ultimately being produced.

**PURPOSE**

The purpose of our study was to characterize cyst morphology using phase contrast microscopy, and the number of precursor cells within each cyst type using nuclear fluorescence staining with Hoechst 33342. The current work is aimed at analyzing spermatogenic cell arrangements in cysts undergoing nuclear transformation using three-dimensional imaging via confocal laser scanning microscopy. Our goal is to develop a reliable system for identifying each cyst type by phase contrast imaging in culture. It is currently unknown how the transforming nuclei are arranged with respect to each in three dimensions, so confocal imaging collects novel 3D datasets that show this relationship. We have already developed a method for mounting cysts in low melting point agarose and preliminary results show that low background 3D datasets are easily collected.

**RESULTS**

![Figure 1: 18 Nuclei Spermatopathea 40x Phase Contrast and Fluorescence](image1)

![Figure 2: 32 Nuclei Spermatopathea 40x Phase Contrast and Fluorescence](image2)

![Figure 3: 32 Nuclei Spermatopathea 40x Phase Contrast and Fluorescence](image3)

![Figure 4: 18 Nuclei Spermatopathea 40x Phase Contrast and Fluorescence](image4)

![Figure 5: 64 Nuclei Secondary Spermatocyte Phase Contrast and Fluorescence](image5)

![Figure 6: 64 Nuclei Secondary Spermatocyte Phase Contrast and Fluorescence](image6)

![Figure 7: Elongating Cysts Phase Contrast and Fluorescence](image7)

![Figure 8: Mature cyst intensity projections of confocal image stacks](image8)

Figs. 1 - 7 above show two phase contrast and two fluorescent images of each stage.

**MATERIALS AND METHODS**

**Tests Dissection and Cyst Release**

Our experimental approach for obtaining spermatogenic cysts, both for 3-D imaging and in vitro culture is as follows: Pupae are soaked in 70% ethanol for 15 minutes and the flies are then dissected from the pupal cases on a sterilized bench. Three sets of testes are removed from three flies and are torn open in 1X PBS with forces sterilized with ethanol, releasing the cysts. The cysts are dissociated by carefully teasing them apart with sterilized forceps in a 995µL PBS 5µL Hoechst solution. The cysts sit in the PBS/Hoechst solutions for 5 minutes after being dispersed to allow the Hoechst to penetrate the cells.

**Phase-contrast, Fluorescence, and Confocal Imaging**

For 3-D confocal imaging, the cysts are gently mixed with 0.5% low melting point agarose, 5 ug/ml Hoechst 33342 solution and allowed to solidify at room temperature. These mounts were imaged on an Olympus FV 1000 confocal microscope, and the data analyzed and reconstructed with Bitplane Imaris. Wide-field fluorescence and phase-contrast imaging was done on a Leica DMIL inversed microscope.

**SUMMARY**

We have analyzed spermatogenic cyst morphology to accurately characterize cyst maturation in our in vitro culture system. Phase contrast and fluorescence pictures were taken capturing all stages of cysts cell maturation. Through the course of the semester, a large collection of each stage has been created to create a standard method in further cyst cell analysis. Accurate characterization of cyst maturation in in vitro spermatogenic culture will improve our ability to quantify cyst survival under different treatment conditions.

**FUTURE WORK**

Based on the characterization of cyst morphology, further research involves the development of a method for visualizing nuclear transformation in real-time using our previously developed culture system. We propose to test the effects of two DNA-specific dyes on cell viability: Hoechst 33342, and Syto-Red. Each of these dyes will stain living cells and recent work indicates that Hoechst 33342 is non-toxic in time-lapse live cell imaging when dye concentration and excitation frequency is limited (Purschke et al., 2010).

**REFERENCES**


---

**Spermatogenesis in *D. pseudoobscura***

Representation of spermatogenesis in *D. pseudoobscura*. (a) The stem cell niche showing the progression (left to right) of the encapsulation of a gonialblast (Hb cells, GSC gonadal stem cell, MSC somatic stem cell or cyst progenitor cell). (b) Overview view of the testes showing the stages of cyst development. In *D. pseudoobscura*, spermatogenesis proceeds only through five divisions to produce 12 cells, which grow in size and replicate their DNA to become primary spermatocytes. Primary spermatocytes enter into meiosis with the first meiotic division resulting in 64 secondary spermatocytes. The final meiotic division results in 128 round haploid spermatozoa that then transform into mature spermatozoa during spermiogenesis and burst from the encapsulated cyst. As elongation proceeds, the sperm heads move toward the basal end of the cyst. The mature spermatozoa enter the seminal vesicle and are stored until mating (SG spermatocytes, P primary spermatocyte, S SP secondary spermatocyte, R round spermatid, E elongating spermatid, MS mature spermatozoa, SC cyst cells). (c) Paired testes, with the accessory glands (AG) attached. Motile spermatozoa stored in the seminal vesicle (SV) until mating (T-test).