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

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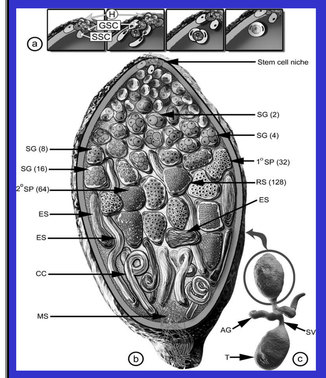
BACKGROUND

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. Germline stem cells are maintained in the stem cell niche in the apical end of the testis. Germline 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 the two meiotic divisions, resulting in 124 haploid cells ultimately being produced.

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 (H hub cells, GSC germline stem cell, SSC somatic stem cell or cyst progenitor cell). (b) Cut-away view of the testes showing the stages of cyst development. In D. pseudoobscura, spermatogonia proliferate mitotically through five divisions to produce 32 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 spermatids that then transform into mature spermatozoa during spermiogenesis and burst from the encapsulating cysts. As elongation proceeds, the sperm heads move toward the basal end of the testis. The mature spermatozoa enter the seminal vesicle and are stored until mating (SG spermatogonia, 1º SP primary spermatocyte, 2º SP secondary spermatocytes, RS round spermatids, ES elongating spermatids, MS mature spermatozoa, CC coiling cyst). (c) Paired testes, with the accessory glands (AG) attached. Mature sperm are stored in the seminal vesicles (SV) until mating (T = testis)



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 3-D 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 3-D datasets are easily collected.

RESULTS

Figure 1. 8 Nuclei Spermatogonia
40x Phase Contrast and Fluorescence

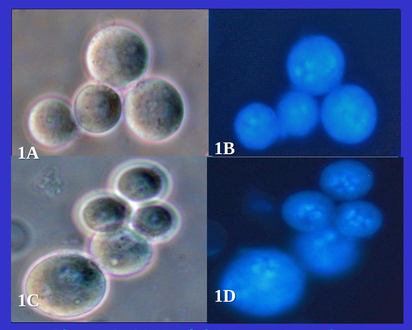


Figure 2. 16 Nuclei Spermatogonia
40x Phase Contrast and Fluorescence

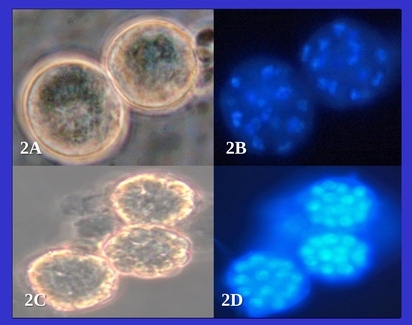


Figure 3. 32 Nuclei Spermatogonia
40x Phase Contrast and Fluorescence

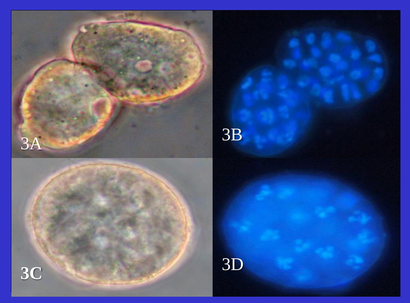


Figure 4. 32 Nuclei Primary Spermatocytes
40x Phase Contrast and Fluorescence

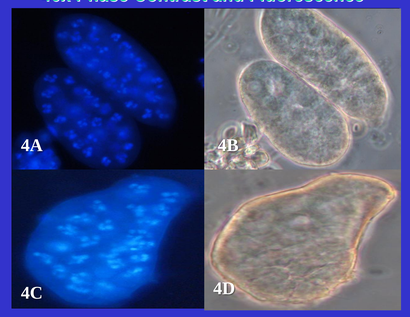


Figure 5. 64 Nuclei Secondary Spermatocyte
Phase Contrast and Fluorescence

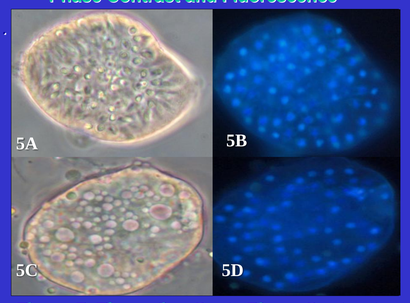


Figure 6. 128 Nuclei Round Spermatid Phase
Contrast and Fluorescence

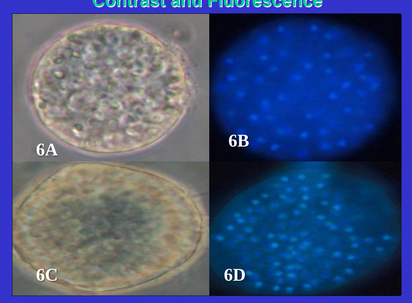
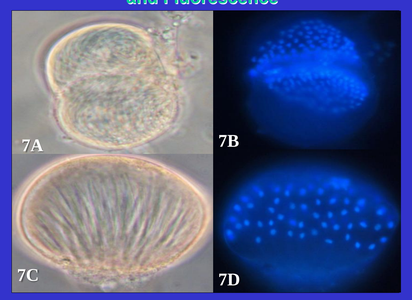
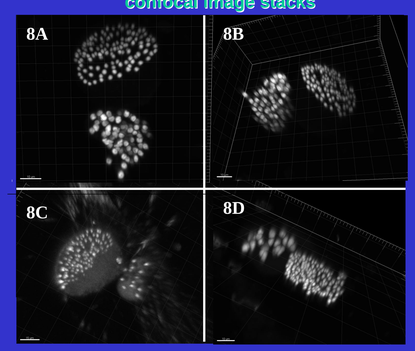


Figure 7. Elongating Cysts Phase Contrast
and Fluorescence



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

Figure 8. Maximum intensity projections of
confocal image stacks



MATERIALS AND METHODS

Testes 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 forceps 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 inverted 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).

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