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Review

Studying how flies make sperm—Investigating gene function in *Drosophila* testes

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ABSTRACT

The majority of the audience at the European Testis Workshop were actively researching mammalian spermatogenesis or sperm function. In this paper therefore I follow the brief I had for my contribution to the meeting in the session "non-mammalian models of spermatogenesis", which was to explain practical approaches to research in *Drosophila* spermatogenesis, illuminating how what we learn in model organisms can be relevant to male fertility research in mammals. I discuss techniques and resources available to fly spermatogenesis researchers, and indicate how I have applied them in my lab to understand regulation of gene expression in spermatogenesis.

Contents

1. Why and how do we study spermatogenesis in *Drosophila*?

1.1. Why spermatogenesis?

Sperm are highly specialised cells with several unique morphological and cellular characteristics—the acrosome for example is sperm specific, while sperm chromatin packaging is completely different from packaging of DNA in other cell types. Sperm have organelles and structures in common with somatic cells, but often with a sperm-specific elaboration. The flagellar axoneme is very similar to the axoneme of motile cilia, but has some sperm-specific components. Similarly the mitochondria in sperm adopt a specialised morphology. Thus it is clear that spermatogenesis, i.e. the making of sperm, requires coordinated action of many different cell biological events to carry out a cell-type specific differentiation process of great complexity. Sperm production continues throughout the adult life of most male animals. Continued supply of precursor cells is essential for this, and so spermatogenesis is typically maintained via a stem cell system. On average, each stem cell division results in another stem cell and a spermatogonial cell committed to differentiation. This spermatogonial cell undergoes a limited number of mitotic amplification divisions before exiting the mitotic cell cycle and committing to the meiotic programme as a primary spermatocyte.

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Studying sperm formation therefore will illuminate understanding of basic cell biological processes, including membrane remodelling, mitochondrial morphogenesis and axoneme formation, among others. Spermatogenesis research is also relevant to understanding cell behaviour in a developmental context, particularly stem cell maintenance and promotion of cellular differentiation.

1.2. Why Drosophila spermatogenesis?

Drosophila melanogaster has a long and distinguished history as a genetic model organism for the study of developmental and cell biological processes. The short generation time, relative ease of genetic manipulation, availability of genetic resources and fully sequenced genome make this an attractive system for study. Spermatogenesis in *Drosophila* is relatively simple, dispensable for adult viability, and amenable to genetic, cell biological and biochemical investigation. The stages of spermatogenesis are well defined, cells are large and easily accessible, and there is evidence that many aspects of the genetic control of spermatogenesis are conserved. Spermatogenesis follows a multi-step differentiation programme involving dramatic changes in cell cycle dynamics, gene expression and morphogenesis (for reviews of the process see (Fuller, 1993; Renkawitz-Pohl et al., 2005)). The *D. melanogaster* testis is a blind-ended tube made of muscle and pigment cells on the outside, and male germline and somatic support cells within the testis proper. At the closed (apical) end of the 2 mm long testis is a specialised cluster of post-mitotic somatic cells known as the hub. Clustered in a rosette around the hub are two stem cell populations—male germline stem cells (GSC) and somatic cyst progenitor cells (CPC). Birth of new cells in this germinal proliferative centre results in displacement of older cells along the testis, leading to a spatio-temporal array of spermatogenic stages visible in every adult testis (Fig. 1A). After the stem cells divide the spermatogonial daughter is encapsulated by two cyst cells, daughters of two CPCs. Both stem cell populations rely on signals from the hub for their maintenance, and they are interdependent for regulation of normal proliferation and differentiation. Cell–cell

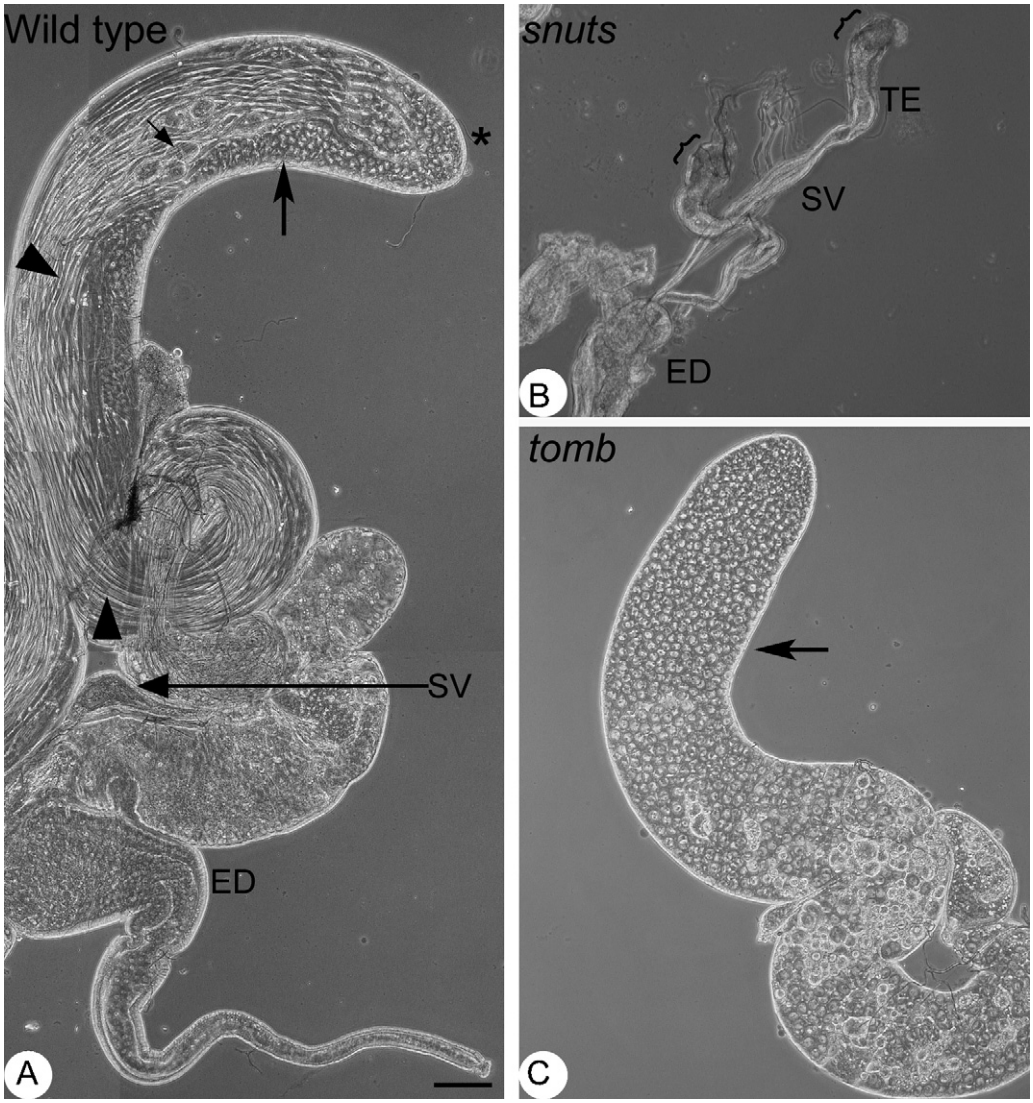

Fig. 1. Phase contrast micrographs of wild type and mutant *Drosophila melanogaster* testes. (A) A wild-type *Drosophila* testis and portion of male genital tract, viewed live after gentle squashing with a cover-slip. The apical region of the testis, where stem cells reside, is at the top marked by an asterisk. Primary spermatocytes are visible as large round cells near the apical region (large arrow). Elongating spermatid tails resemble ropes pushing up the lumen of the coiled testis (arrowheads). Waste bags, containing cytoplasm extruded during individualisation, are found in the apical region of the testis (small arrow). (B) *snuts* testes (brackets), are dramatically smaller than wild type, and are shown still attached to the terminal epithelium (TE) seminal vesicles (SV) and anterior ejaculatory duct (ED). (C) *tomb* mutant testes show a typical meiotic arrest phenotype, with morphologically normal primary spermatocytes (arrow), but no progression into meiosis or into spermatid differentiation. Scale bar is 100 μm and applies to all panels.