Video Article

Cytological Analysis of Spermatogenesis: Live and Fixed Preparations of *Drosophila* Testes

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Abstract

Drosophila melanogaster is a powerful model system that has been widely used to elucidate a variety of biological processes. For example, studies of both the female and male germ lines of *Drosophila* have contributed greatly to the current understanding of meiosis as well as stem cell biology. Excellent protocols are available in the literature for the isolation and imaging of *Drosophila* ovaries and testes[3-12]. Herein, methods for the dissection and preparation of *Drosophila* testes for microscopic analysis are described with an accompanying video demonstration. A protocol for isolating testes from the abdomen of adult males and preparing slides of live tissue for analysis by phase-contrast microscopy as well as a protocol for fixing and immunostaining testes for analysis by fluorescence microscopy are presented. These techniques can be applied in the characterization of *Drosophila* mutants that exhibit defects in spermatogenesis as well as in the visualization of subcellular localizations of proteins.

Video Link

The video component of this article can be found at https://www.jove.com/video/51058/

Introduction

Drosophila testes are an ideal model system for the study of many biological processes including the regulation of stem cells, meiosis, and sperm development[13-18]. The spermatocytes and their meiotic spindles are large and hence convenient for cytological analysis, and relaxed cell cycle checkpoints during spermatogenesis facilitate the study of mutations in cell cycle genes. Different cell types can be observed in ordered progression along the length of the testes, and any disruption in spermatogenesis can lead to changes in this overall arrangement. These features combined with *Drosophila* genetic tools have facilitated the mutational analysis of spermatogenesis[21-23].

The stages of *Drosophila* spermatogenesis have been well defined. Germline cells that develop synchronously within cysts progress sequentially through the stages of spermatogenesis along the length of the testis. During both the mitotic and meiotic divisions of the male germ cells, cytokinesis occurs incompletely such that the daughter cells remain connected by cytoplasmic bridges known as ring canals (**Figure 1**). The apical tip of the testis contains a population of germline stem cells that gives rise to spermatogonial cells, which undergo four mitotic divisions with incomplete cytokinesis to generate 16-cell cysts of primary spermatocytes. After premeiotic S phase, primary spermatocytes enter G2, a prolonged growth period of ~90 hr during which cellular volume increases ~25-fold. Progression through meiosis I and meiosis II results in the formation of 32-cell cysts of secondary spermatocytes and 64-cell cysts of haploid spermatids, respectively. The immature, round spermatids undergo extensive cellular remodeling to form mature sperm. Post-meiotic cells, in particular the bundles of elongating and mature spermatids, occupy much of the volume of the testis.

The successful transport of functional sperm to female flies requires coordination between the different parts of the male reproductive system, which is composed of several paired structures (the testes, seminal vesicles, and accessory glands) and a single ejaculatory duct (**Figure 2**). Sperm are produced within the testes and stored within the seminal vesicles until copulation[24]. The accessory glands contain secretory cells that produce seminal fluid. The sperm migrating from the seminal vesicles are mixed with seminal fluid within the ejaculatory duct, which is connected to both the seminal vesicles and the accessory glands. This mixture of sperm and seminal fluid is ultimately pumped out of the male into the vagina of the female fly through the ejaculatory bulb located at the posterior end of the male abdomen[25]. Proteins within the seminal fluid are essential for prolonged storage of sperm within specialized organs known as spermathecae in the reproductive tract of *Drosophila* females[26].

Excellent methods for the isolation of *Drosophila* testes and visualization of cells at various stages of spermatogenesis are available in the scientific literature[3-12]. We herein add to this body of knowledge by presenting examples of these protocols with an accompanying video demonstration. The protocol for preparation of live testes samples for phase-contrast microscopy is based on a previously described method[27]. The protocol for formaldehyde fixation and immunostaining of testes is also based on a previously described method[28]. The approaches described herein have been used in many studies of *Drosophila* spermatogenesis (for example, to assess the roles of dynein, a minus-end-directed microtubule motor, during *Drosophila* spermatogenesis).

In addition to the basic protocols, suggestions are provided for varying the dissection so as to enrich for spermatogonia, spermatocytes, or mature sperm. Different methods for processing the testes such that cysts either remain intact or are disrupted as needed are described. An advantage in using *Drosophila* testes as a model system is that, compared to *Drosophila* oocytes and embryos, antibodies and dyes can easily penetrate cells following their dispersal from the testes, and fewer washing steps are required; thus, protocols can be performed in a relatively short time.

Protocol

1. Testes Dissection

1. Anesthetize flies in a bottle or vial using a stream of CO_2 and transfer to a fly pad.
2. Sort flies under a dissecting microscope using a small paintbrush, and collect an appropriate number (depending on the experiment) of *Drosophila* males of the desired genotypes. Young males (0-2 days old) are ideal for examining cells throughout the earlier stages of spermatogenesis (*e.g.* spermatogonia, spermatocytes, and early post-meiotic spermatids), whereas slightly older males (2-5 days old) are ideal for examining cells in the final stages of spermatogenesis (in particular, mature sperm).
3. Use forceps to remove the wings from each fly (to prevent the flies from floating in liquid during dissection).
4. Add ~500 ml of phosphate-buffered saline (130 mM NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4; PBS) in a drop to a silicone-coated dissection dish on a black background. Other aqueous solutions have been successfully used for testes dissection[3].
5. Point forceps towards the anterior of the fly, grasp it by the thorax, and immerse it in the PBS drop. While viewing through the dissecting microscope, use another pair of forceps to grasp and pull the external genitalia (dark brown structure located at the posterior end of the ventral abdomen) posteriorly until it detaches from the abdomen. In most cases, the testes, seminal vesicles, and accessory gland will be removed from the abdomen along with the external genitalia; if not, insert a single pair of forceps into the abdomen and tease out the testes.
6. Separate testes from accessory gland and external genitalia using two pairs of forceps in the PBS drop (**Figure 2**). Wild-type testes are easily distinguished from neighboring white tissues by their yellow color. Proceed immediately to step 2.
7. To isolate testes from pharate males (*i.e.* enclosed within the pupal case), an additional step must first be performed that involves removing the fly from the pupal case; this step has been previously described elsewhere[31]. Proceed with dissection as for the adult testes beginning at step 1.2.
8. To isolate testes from larval males, perform a modification of a protocol for isolating *Drosophila* larval ovaries[32]. Briefly, male larvae can be distinguished from female larvae by the presence of a pair of large, clear, oval structures (larval testes) embedded in the posterior third of the fat body. To isolate larval testes, partially flay open the male larva to isolate the testes and the surrounding fat body from the abdomen as described for the isolation of larval ovaries. Proceed immediately to step 2 of the protocol described herein; the testes can later be removed from the fat body just prior to mounting (steps 2.3 or 3.14) as described for the ovaries[32].

2. Sample Preparation and Live Imaging

1. Use a pair of forceps to gently place 2-3 pairs of testes in a drop of 4-5 ml of PBS on a square glass cover slip. Note that the ratio of testes number to PBS volume may need to be adjusted: too much liquid will prevent cells from spreading properly when squashed, whereas too little liquid will cause cells to burst when squashed. Optional: Use siliconized cover slips to minimize adherence of tissue to cover slip in step 3.2.
2. Use a pair of forceps to tear open each testis at an appropriate position so as to maximize the presence of the desired germline cell types in the preparation (note that the contents of the testis will mostly egress from the torn region onto the slide during the squashing step.) To enrich for spermatogonia and spermatocytes, tear open the testis adjacent to its apical tip (level 1, **Figure 2B**). To enrich for spermatocytes and spermatids, tear open the testis at a position slightly basal to level 1 (level 2, **Figure 2B**). To enrich for more mature germline cells, tear open the testis closer to where the curvature begins (level 3, **Figure 2B**).
3. Gently place a glass microscope slide over the cover slip to squash the testes; do not apply pressure manually as the weight of the cover slip alone is sufficient to obtain a properly squashed sample. Try to avoid trapping air bubbles. Optional: Use poly-L-lysine coated microscope slides to promote adherence of tissue to slide in step 3.2.
4. Use preparation immediately (ideally within 15 min of preparation) to observe live cells by phase-contrast microscopy; for transgenic flies with expression of fluorescently tagged proteins in the testes, live cells can be examined by fluorescence microscopy at this step. Alternatively, proceed with fixation and antibody staining (Protocol 3).
5. Gently wick any excess liquid from under the coverslip using a cleaning wipe to allow flattening of the preparation until the germ cells are clearly in focus.

3. Formaldehyde Fixation and Antibody Staining

1. Snap freeze each slide containing squashed testes (from Protocol 2) using a pair of metal tongs to immerse it briefly in liquid nitrogen (until liquid nitrogen stops bubbling).
2. Remove the cover slip immediately using a razor blade.
3. Use metal tongs to transfer slides to a prechilled glass slide rack filled with ice-cold 95% ethanol (spectrophotometric grade, methanol-free). Store at -20 °C for 10 min.
4. Use metal tongs to transfer slides to a glass slide rack filled with 4% formaldehyde in PBS plus 0.1% Triton X-100 (PBS-T). Store at room temperature for 7 min.
5. Use metal tongs to transfer slides to a glass slide rack filled with PBS. Wash slides in PBS for 5 min at room temperature. Repeat 1x. Perform all washes by discarding solution in the glass slide rack (*i.e.* by pouring it out) and replacing with fresh solution.
6. Discard the PBS and immerse slides in PBS-T for 30 min at room temperature to permeabilize cell membranes.
7. Wash slides in PBS for 5 min at room temperature. Repeat 2x.
8. Blocking step (optional): Immerse slides in PBS plus 1% BSA for 45 min at room temperature.

9. Use a hydrophobic barrier pen to draw a circle on the slide around squashed tissue (easily visible by eye) in order to confine the antibody solutions (added in steps 3.9 and 3.11). The tissue should be kept moist at all times while performing immunostaining.
10. Add 30-40 ml of primary antibody (diluted in PBS-T, 1:400 to 1:50, depending on antibody) to tissue within the circle. If blocking was performed, dilute primary antibody in PBS-T plus 1% BSA. Anti-gamma-tubulin antibody (for staining of centrosomes in **Figure 4**) was diluted 1:100. Incubate in a moist, dark chamber (*e.g.* closed plastic box with damp paper towels) for 2 hr at room temperature or overnight at 4 °C.
11. Wash slides in PBS for 5 min at room temperature 3x. If blocking was performed, wash twice in PBS-T and once in PBS (5 min at room temperature each).
12. Add 30-40 ml of fluorophore-conjugated secondary antibody (diluted 1:400 in PBS) to the tissue and incubate in the dark for 1-2 hr at room temperature.
13. Wash slides in PBS for 5 min at room temperature. Repeat 2x.
14. Add 30-40 ml of DAPI solution (0.2 mg/ml in PBS) to the tissue within the circle.
15. Gently place a glass cover slip over the tissue, taking care to avoid trapping air bubbles. If air bubbles should appear, carefully move around the cover slip without destroying the squash until the bubbles escape from the sides of the cover slip.
16. Use a cleaning wipe to blot excess DAPI from the edges of the slide.
17. Seal the cover slip to the slide using clear nail polish.
18. Use this preparation within the next 3-4 hr to view immunostained cells by fluorescent microscopy. For longer-term storage of slides (up to at least several weeks), use a glycerol-based hard mount media with DAPI, and store slides at -20 ˚C.
19. Alternatively, fix samples in methanol instead of formaldehyde (depending on the antigen). After completing the entire protocol through step 3.2, immerse slides of squashed testes in methanol for 10 min at -20 °C and proceed with step 3.5 onward.

Representative Results

An example of a properly dissected pair of *Drosophila* male reproductive organs is shown in **Figure 2A**. Testes removed from the abdomen of the adult male fly are typically attached to the ejaculatory duct (brown, **Figure 2A'**) and a pair of accessory glands (green, **Figure 2A'**) via a pair of seminal vesicles (blue, **Figure 2A'**). To separate the testes from most of the accompanying somatic tissue, the ejaculatory duct and the accessory glands should be detached and discarded such that only the testes pair and the seminal vesicles remain (**Figures 2B** and **2B'**). The seminal vesicles can also be removed, leaving only the testes pair to be processed further.

The age of the adult males selected for testes dissection is an important consideration. In young males (0-2 days after eclosion), the seminal vesicles are small and nearly empty, and the testes are at their maximal diameter (as in **Figure 2**). Using younger males ensures a relatively large number of dividing germline cells (undergoing either mitosis or meiosis) and early post-meiotic spermatids. When older males (3-5 days after eclosion) are used for dissection (image not shown), the seminal vesicles are more prominent because they are bulging with sperm, and the testes are narrower than in younger males. Older males are preferable if the goal is to visualize mature sperm.

Owing to the ordered progression of developmental events along the length of the *Drosophila* testes, germline cells at specific stages of spermatogenesis can be enriched based on the position at which dissected testes are torn prior to squashing. For example, tearing open the testis near its apical tip (level 1, **Figure 2B'**) yields an abundance of cells in early stages of spermatogenesis. **Figure 3A** shows a phase-contrast image of a representative population of spermatogonia (white arrow), early primary spermatocytes (yellow arrow), and late primary spermatocytes (red arrow) obtained in this way. Alternatively, tearing open the testis at a slightly more basal position (level 2, **Figure 2B'**) yields primarily spermatocytes and spermatids. **Figure 3B** shows a phase-contrast image of a representative cell population of primary spermatocytes (red arrow), round spermatids (green arrow), elongating spermatids (orange arrow), and mature sperm bundles (blue arrow) obtained in this way.

Phase-contrast imaging of testes can be used to characterize defects in *Drosophila* spermatogenesis resulting from mutations in genes that are critical for this process. The round spermatids have a very stereotypical appearance when viewed through a phase-contrast microscope. Each of these immature spermatids has a single, phase-light, round nucleus and a single, phase-dark, round mitochondrial aggregate of roughly the same size (marked by purple arrowhead and arrow, respectively, in **Figure 3C**). Variation in the relative numbers or sizes of these organelles can result from aberrant meiotic divisions (see discussion). An example of such a defect is shown in **Figure 3D**. Round spermatids from adult males with mutation of the *asunder* (*asun*) gene contain a single large mitochondrial aggregate and multiple small nuclei as a result of defects in cytokinesis and chromosome segregation [29].

Fluorescence microscopy is another powerful approach for studying *Drosophila* spermatogenesis. An example of a fluorescent image of fixed cells from a squashed testes sample is shown in **Figure 4**. Testes were obtained from transgenic flies expressing GFP-beta1-tubulin (product of *bTub56D* gene fused at its C-terminal end to GFP and under control of the *Ubi-p63E* ubiquitin gene promoter; gift from H. Oda and Y. Akiyama-Oda, JT Biohistory Research Hall, Osaka, Japan) in order to label the microtubules (green in **Figure 4D**, grayscale in **Figure 4A**). The transgenic testes were immunostained using a mouse antibody against gamma-tubulin (secondary antibody: anti-mouse Cy3) to mark centrosomes (red in **Figure 4D**, grayscale in **Figure 4C**) and DAPI-stained to mark DNA (blue in **Figure 4D**, grayscale in **Figure 4B**). Cells at different stages of spermatogenesis can be readily identified by examining the arrangement of the microtubules, centrosomes, and chromosomes (see legend).

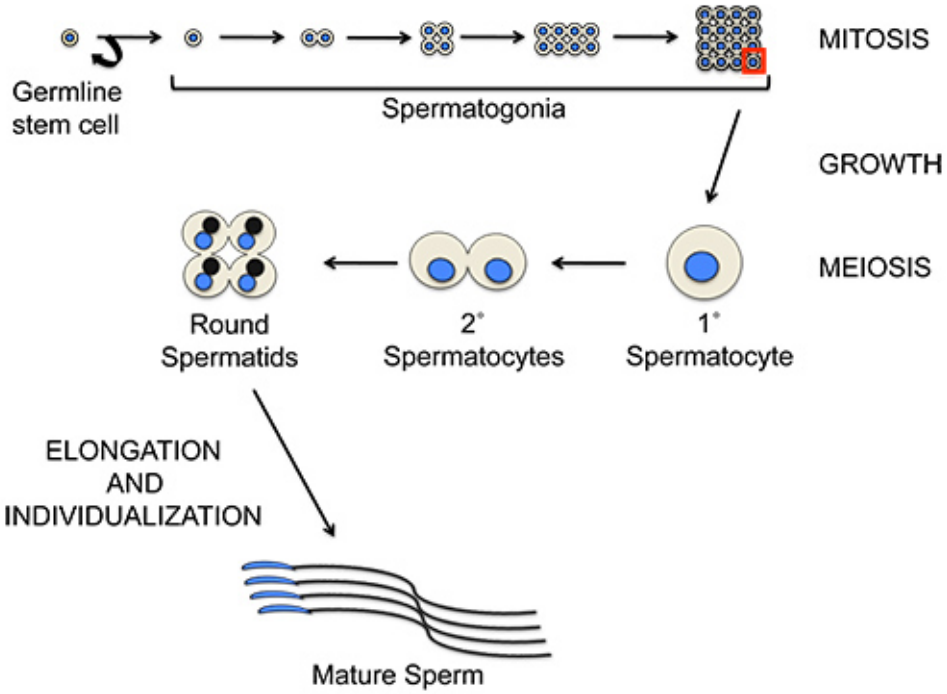

Figure 1. Schematic of germline cell divisions in *Drosophila* males. Each stem cell division produces a gonial cell that undergoes four rounds of mitotic divisions with incomplete cytokinesis to produce a 16-cell cyst of primary spermatocytes. Each primary spermatocyte undergoes a prolonged growth phase prior to undergoing the two meiotic divisions, again with incomplete cytokinesis, to form a 32-cell cyst of interconnected secondary spermatocytes followed by a 64-cell cyst of interconnected round spermatids. Round spermatids are characterized by the presence of a phase-light nucleus and a phase-dark mitochondrial aggregate (the Nebenkern) of similar sizes. The round spermatids undergo elongation and individualization to form the mature sperm. Mature sperm heads can be identified by the their needle-shaped nuclei. Nuclei are shown in blue; Nebenkerne (mitochondrial aggregates) in black; cytoplasm in tan. Click here to view larger image.

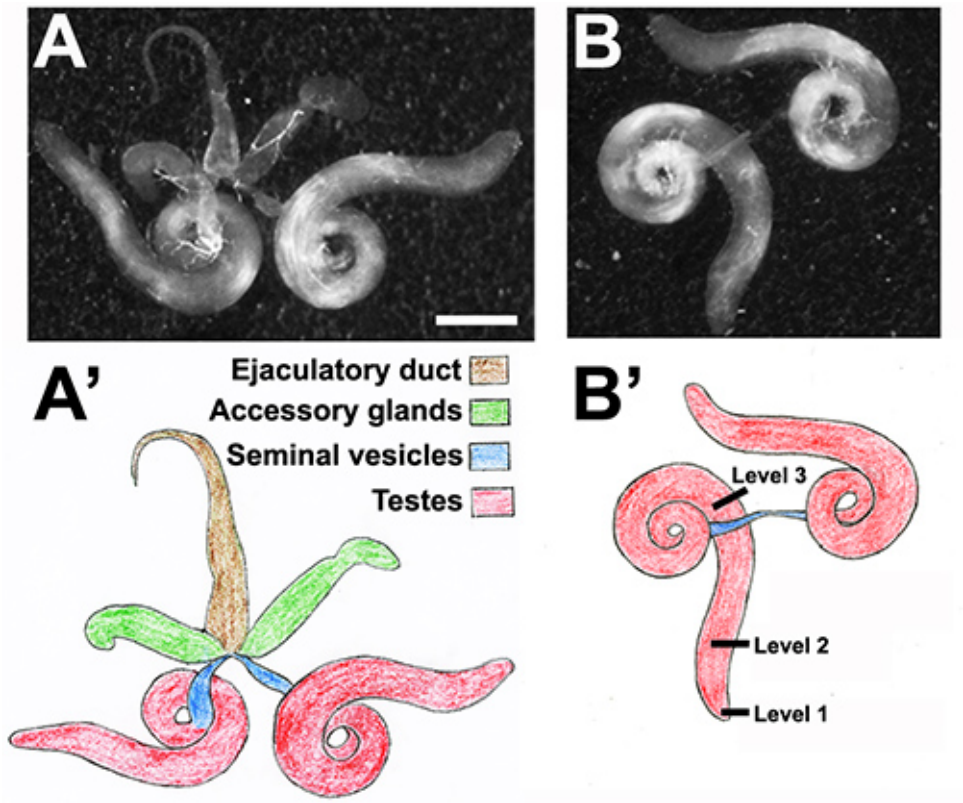

Figure 2. Reproductive organs of *Drosophila* males. Young adult males (0-2 days after eclosion) were selected for dissection. Light micrographs of isolated reproductive organs are shown in **A** and **B** with corresponding cartoons in **A'** and **B'**. (**A, A'**) *Drosophila* testes (red) removed from the abdomen of an adult male fly are connected to the ejaculatory duct (brown) via the seminal vesicles (blue). A pair of accessory glands (green) is also attached to the ejaculatory duct. (**B, B'**) The testes should be separated from the accessory glands before slide preparation. Prior to squashing, tear open the testes at level 1 to enrich for cells at early stages of spermatogenesis; at level 2 to obtain a mixed population of meiotic and post-meiotic germline cells; and at level 3 to enrich for mature sperm. Scale bar, 250 mm. Click here to view larger image.

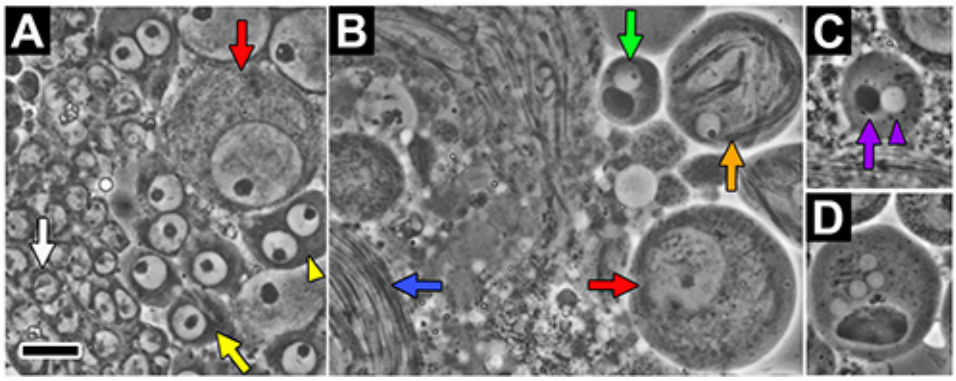

Figure 3. Phase-contrast images of *Drosophila* testes. (**A**) An abundance of cells at early stages of spermatogenesis, including spermatogonia (white arrow), early primary spermatocytes (yellow arrow), and late primary spermatocytes (red arrow) are typically released when the testis is torn at level 1 (see **Figure 2B'**). Two or more interconnected cells (a consequence of incomplete cytokinesis) often fuse completely as an artifact of the squashing procedure (yellow arrowhead marks a fusion of two early spermatocytes). (**B**) A combination of spermatocytes (red arrow marks late primary spermatocyte) and post-meiotic cells, including round spermatids (green arrow), elongating spermatids (orange arrow marks partial cyst), and mature sperm bundles (blue arrow), are typically released when the testis is torn at level 2 (see **Figure 2B'**). (**C**, **D**) Phase-contrast imaging of squashed testes can be used to readily identify defects in the abundant and stereotypical round spermatids. Wild-type spermatids have one nucleus (phase-light; marked by purple arrowhead) attached to a single mitochondrial aggregate (phase-dark; marked by purple arrow) of roughly equal size (**C**). Spermatids from *asunf02815* testes contain multiple small nuclei and one large mitochondrial aggregate as a result of failed cytokinesis and errors in chromosome segregation (**D**). Scale bar, 10 mm. Click here to view larger image.

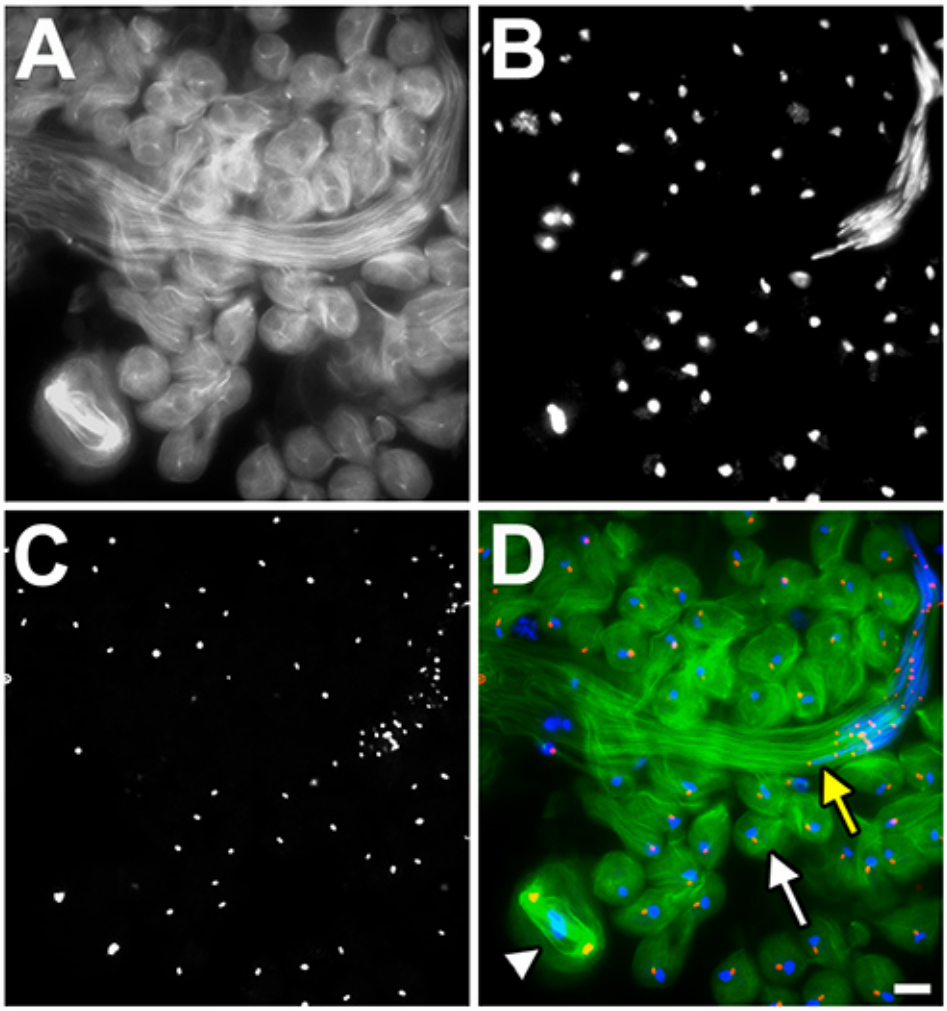

Figure 4. Fluorescent image of *Drosophila* testes. Preparations of squashed testes isolated from males expressing GFP-tagged beta1-tubulin (green in **D**, grayscale in **A**) were fixed and stained for both DNA (DAPI; blue in **D**, grayscale in **B**) and centrosomes (gamma-tubulin antibody; red in **D**, grayscale in **C**). A dividing spermatocyte (white arrowhead) can be seen next to a large field of round spermatids (white arrow marks a representative cells) and a bundle of mature sperm (yellow arrow). Scale bar, 10 mm. Click here to view larger image.

Discussion

Although the testes of wild-type flies can be readily identified due to their yellow color (in contrast the neighboring white tissues), the testes of *white* mutant flies are white and thus can occasionally be confused with the gut. Most transgenic strains, which are typically in a *white* background, also have white testes because the mini-*white* gene found in *P*-elements does not promote pigment accumulation in the testes. When *Drosophila* testes cannot be distinguished by color, other easily recognizable features include their spiral pattern and occurrence in pairs[12]. Note that some workers find it easier to use dissection needles instead of forceps to isolate the testes[12].

A critical step of this protocol is the preparation of the squashed testes. One useful approach is to hover the slide a few millimeters over the cover slip containing the testes such that the surface tension of the PBS on the cover slip lifts up the cover slip containing the tissue towards the slide. This method tends to disrupt cysts and spread out cells on the slide, making it ideal for imaging individual cells. Alternatively, to maintain entire or partial cysts for imaging, the volume of PBS placed on the cover slip can be increased (from 4-5 ml to 7-8 ml).

If a particular *Drosophila* mutant of interest does not survive to adulthood, then larval or pupal testes can alternatively be used to study spermatogenesis. In the protocol section, references are provided for the isolation of pharate males and larval ovaries; a modification of the latter protocol can be used to isolate larval testes. The steps for tearing, squashing, and staining larval or pupal testes are essentially identical to that described herein for the adult testes. Even if the fly line to be studied can reach adulthood, isolation of larval or pupal testes may be preferable if the goal is to obtain cells at the earliest stages of spermatogenesis. As described in the representative results section, cells in early or late stages of spermatogenesis can be enriched as desired by varying the level at which the testis is torn prior to squashing.

Phase-contrast microscopy offers a relatively simple approach for studying *Drosophila* spermatogenesis. One advantage of phase-contrast microscopy over immunostaining and fluorescent microscopy is that less time is required to prepare the samples. All of the major stages of spermatogenesis can be readily identified using this technique[24]. Indeed, several groups have used phase-contrast microscopic analysis of testes as a primary screen for characterizing the phenotypes of *Drosophila* male-sterile mutant lines[21-23]. During meiotic divisions, mitochondria

and chromosomes are equally partitioned to daughter cells. A unique feature of spermatogenesis in *Drosophila* and other insects involves formation of a mitochondrial aggregate (the Nebenkern) in round spermatids[24]. Round spermatids contain a phase-dark Nebenkern and a phase-light nucleus of roughly equal size in a 1:1 ratio (**Figure 3**). The diameter of the nucleus reflects the DNA content of the round spermatids, so errors in chromosome segregation during meiosis can lead to variability in nuclear size[33]. Disruption of meiotic cytokinesis following chromosome segregation results in multinucleated round spermatids in which the Nebenkerne fuse into a single aggregate[34]. Hence, any variation in the 1:1 ratio or in the size or shape of the Nebenkern and nucleus in round spermatids can be readily detected by phase-contrast microscopy of live testes and is often diagnostic of defects in meiosis. Fusion of two or more interconnected cells within a common cyst frequently occurs as an artifact of the squashing procedure (**Figure 3A**, yellow arrowhead); the 1:1 ratio of nuclei to Nebenkerne, however, is still maintained.

Immunostaining of fixed preparations of *Drosophila* testes can be performed to stage cells undergoing spermatogenesis as well as to assess expression patterns and subcellular localizations of proteins of interest. A refined scheme for classifying the stages of *Drosophila* spermatogenesis based on the cellular morphology, chromatin organization, and microtubule arrangements of testes cells immunostained for tubulin and DNA-stained has been described[19]. *Drosophila* primary spermatocytes are relatively large cells, and the meiotic spindles can be clearly observed using this approach. Coimmunostaining for centrosomes (for example, using antibodies against gamma-tubulin) can be performed to obtain a more precise view of the meiotic spindle structure. Formaldehyde is a suitable fixative when immunostaining testes with a wide range of antibodies (for example, anti-lamin and anti-dynein heavy chain). The morphology of microtubules and centrosomes, however, is better preserved with methanol; hence, methanol is typically used as the fixative when performing immunostaining with antibodies against alpha-tubulin and gamma-tubulin.

If antibodies against a protein of interest are unavailable, transgenic *Drosophila* lines expressing a fluorescently tagged (*e.g.* mCherry or GFP) version of the protein can be generated as an alternative approach. The subcellular localization of the protein can then be determined by using a microscope to view the GFP fluorescence in live or fixed preparations of tissue; alternatively, anti-GFP antibodies can be used to detect the fusion protein in fixed samples. In **Figure 4**, microtubules in a fixed testes preparation were visualized via the intrinsic fluorescence of GFP-tagged beta1-tubulin (expressed from a transgene under control of a globally expressed ubiquitin promoter). Alternatively, transgenic flies in which expression is under the control of a tissue-specific promoter; for example, the promoter of the *beta2-tubulin* gene have been used for testes-specific expression[35]. Alternatively, the expression of a protein of interest can be restricted to specific male germline cells by using the UAS-Gal4 system[36]. *nanos*-Gal4 can be used to induce the expression of a protein under the control of a UAS promoter within spermatogonial cells, while *bam*-Gal4 can be used to induce its expression within spermatocytes[37]. Knockdown of a protein of interest can also be induced in the testes by expressing an RNAi hairpin construct under the control of a UAS promoter in conjunction with these Gal4 drivers[37].

The development of methods for live-image analysis of *Drosophila* testes cells has expanded the range of questions that can be addressed using this system. The events of meiotic cytokinesis can be visualized by phase-contrast microscopy of spermatocytes cultured in fibrin clots inside a perfusion chamber in order to prolong the life of the cells[35]. Time-lapse confocal microscopy of *Drosophila* spermatocytes expressing fluorescently tagged proteins has also been a powerful approach (for example, to study meiotic spindle assembly)[38]. The use of confocal microscopy for live imaging of an earlier stage, the dividing germline stem cells of the *Drosophila* testes, has led to an increased understanding of stem cell regulation. In addition to the phase-contrast and immunofluorescence microscopy approaches presented herein, transmission electron microscopy has also been used extensively to study *Drosophila* spermatogenesis[31].

In addition to imaging, *Drosophila* testes can be used as a source of material for biochemical analysis. For immunoblotting experiments, dissected fly testes can be homogenized in 6x sample buffer (5 ml/testes pair), boiled, and directly loaded on a SDS-PAGE gel (~4 testes pairs/lane). For example, this approach has been used to assess the levels of dynein-dynactin components in the testes of several *Drosophila* mutants. Testes extracts can alternatively be prepared by homogenizing the tissue in nondenaturing lysis buffer if it is important to maintain protein integrity. For example, *Drosophila* testes extracts have been used in coimmunoprecipitation experiments to demonstrate the presence of stable protein complexes in this tissue[41-43].

Disclosures

The authors declare that they have no competing financial interests.

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