

Peacock, W.J. and G.L.G. Miklos. CSIRO, Canberra, Australia and Australian National University, Canberra, Australia. Light microscope analysis of spermiogenesis in *Drosophila melanogaster* males exhibiting meiotic drive.

The electron microscope studies of Tokuyasu et al. (1972a, b) have revealed many of the processes involved in normal spermiogenesis in *D. melanogaster*. These authors also described the modifications of some of these processes which occur in Segregation Distorter heterozygotes and homozygotes and we have recently described similar events in the sc^4sc^8 meiotic drive

system (Peacock et al. 1973). We have now found that it is possible to detect a number of these normal and abnormal spermiogenic processes in light microscope preparations. Since the time involved in slide preparation for light microscopy is small relative to that needed for electron microscope analysis, we consider that this technique may be useful in characterising spermiogenic lesions in *Drosophila* stocks.

Methods: Testes of males (usually 0-24 hours old) are dissected in an aqueous solution of 0.7 percent NaCl, the accessory glands removed and the testes transferred to freshly mixed glacial acetic acid: absolute ethanol (1:3). Testes can be stored at -20°C in fixative for extended periods if necessary. The fixed testes are then placed in IN HCl at 60°C for 5 minutes, stained for 30 minutes with feulgen, and examined in 45 percent acetic acid with phase optics. For most observations very little pressure need be applied to the coverslip. All of the solution transfers may be conveniently carried out with a hypodermic syringe to avoid mechanical damage to the testes.

Results: The arrangement of the various developmental stages within the testis is readily seen in a favourable squash. Gonial mitoses in the apical region are sometimes visible and meiotic cysts are frequently observed in the mid-region of the testis. The head regions of the majority of spermatid cysts are grouped in the basal portion of the testis with the tail regions of the cysts extending the full length almost to the apex. Relatively few groups of heads are seen in the mid-region and the ones that do occur there are in early stages of spermatid development. It is thus possible to quantitate the developmental and spatial distribution of cysts in the testis. All these observations are in accord with the descriptions from the electron microscope studies.

Tokuyasu et al. (1972a) described the process of individualisation whereby each of the 64 spermatids, which have developed in the germinal syncytium, are enclosed with a membrane. This process occurs after spermatid elongation. Individualisation is initiated in the head region of the cyst and proceeds caudally. The diameter of the spindle-shaped individualisation bulge (Figure 1) increases as the bulge proceeds along the cyst (Figure 2), the increase being due to the accumulation of cytoplasmic debris. Under the light microscope the individualisation bulge is clearly discernible, and the individualised spermatids (basal to the bulge) and syncytial spermatids (apical to the bulge) have distinctive appearances. The bulge eventually forms a "waste bag" in the apical region of the testis. This too is visible in the light microscope as a patchy, refractile structure near the gonial cysts (Figure 3).

One of the more remarkable processes of spermiogenesis is the entrapment of the head region of each cyst by the cells of the terminal epithelium in the base of the testis (Tokuyasu et al. 1972b). This head trap is not often obvious under the light microscope with the present technique, but can occasionally be seen (Figure 4). More frequently, in developing cysts, the relation of the head cyst cell to the spermatid heads is very clear (Figure 5).

Once the bundle of 64 individualised spermatids is firmly anchored in the terminal epithelium, the entire linear bundle is "reeled in" during the process of coiling. As coiling proceeds, the cyst is distended in the basal region and the waste bag is withdrawn from the apex down the full length of the testis. It is at this stage of spermiogenesis in the SD/SD⁺ and sc^4sc^8 meiotic drive systems that developmental lesions are first obvious under the light microscope. Spermatids which have failed to be individualised are not coiled in the regular manner of individualised spermatids but are loosely folded into the descending waste bag (Figure 6). This figure also shows that non-individualised, or syncytial, spermatids are also visible in the linear cyst because of their increased refractivity and granular appearance. In control males where there are no abnormal spermatids, waste bags are very difficult to see at the base of the testis (Figure 7), and in fact there is a progressive loss of the patchy appearance as it moves down the length of the testis. However in both SD heterozygotes and sc^4sc^8 males waste bags containing abnormal spermatids in various stages of breakdown can be seen in the base of the testis (Figures 8 and 9). Although we have not been able to quantitate this breakdown in respect to the extent of meiotic drive in a particular male, there is a clear distinction between meiotic drive and control males.

Many modifications can be made to this technique. We have found it convenient to make an initial analysis as to the presence or absence of syncytial spermatids, inclusion of syncytial spermatids into descending waste bags, presence or absence of degenerating spermatids in the base of the testis and then to squash more heavily in order to do a detailed analysis of meiotic cysts in the same testis.

This technique should prove useful in surveys for cases of abnormal spermatid development and for characterising stocks showing meiotic drive and partial or complete sterility.

References: Peacock, W.J., G.L.G. Miklos and D.G. Goodchild 1973, Sex chromosome meiotic drive systems in *Drosophila melanogaster*. I. Abnormal spermatid development in males with a heterochromatin deficient X chromosome (sc^4sc^8). Submitted to Genetics; Tokuyasu, K.T., W.J. Peacock and R.W. Hardy 1972a, *Z. Zellforschung Mikroskopische Anatomie* 124:479-506; 1972b, *Z. Zellforschung Mikroskopische Anatomie* 127:492-525.

Figures 1-5 on Facing Page

- Fig. 1. Individualisation bulge (IB) in the lower one third of the testis proceeding along a cyst toward the apex of the testis and leaving behind it individualised spermatids (IS) whilst accumulating cytoplasmic and nucleoplasmic debris.
- Fig. 2. A larger individualisation bulge in the upper one third of the testis showing a more granular appearance of the contents.
- Fig. 3. A waste bag (WB) of debris of the apex at the testis. Although now separated from the spermatid tails, the waste bag is still within the cyst lumen.
- Fig. 4. Basal region of the testis showing coiled spermatid bundles (CB) with the head region of one cyst embedded in the terminal epithelium, or head trap (HT).
- Fig. 5. Mid region of the testis showing an immature cyst of spermatids with syncytial tails (ST) with the heads embedded in the head cyst cell (HC). The head cyst cell-spermatid head region of the cyst will subsequently move basally and be involved in the head trap with terminal epithelial cells.

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- Fig. 6. Mid region of the testis of a sc^4sc^8/y^+Y male exhibiting meiotic drive. A spermatid bundle is in the process of coiling. The individualised spermatids (IS) are distinguishable from the abnormal syncytial spermatids (SS) which have a granular and irregular appearance. The syncytial spermatids terminate in the waste bag (WB) and are folded irregularly into the waste bag as it is drawn basally down the testis during coiling of the normal spermatids.
- Fig. 7. Portion of the reproductive apparatus of a control male (y/y^+Y) showing coiled bundles of sperm (CB) in the base of the testis, the testicular duct (TD) and the seminal vesicle (SV) packed with masses of mature sperm (MS). It should be noted that no refractile bodies are visible near the coiled sperm bundles in the base of the testis.
- Fig. 8. Portion of the reproductive apparatus of a Segregation-Distorter heterozygote. Coiled bundles of sperm (CB) are seen in the base of the testis along with degenerating spermatids (DS). These refractile bodies represent waste bags containing abnormal nonindividualised spermatids.
- Fig. 9. High power shot of the basal testicular region of a sc^4sc^8/y^+Y male exhibiting meiotic drive. The degenerating spermatids (DS) are easily recognizable and are an unmistakable indication of abnormal spermatid development. A coiled bundle of sperm (CB) has its heads clearly visible (SH).



