Nuclear Shaping during Spermiogenesis in the Whip Scorpion

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The mature spermatozoon of the whip scorpion or vinegaron Mastigoproctus giganteus is a spherical cell within which the nucleus and flagellum are tightly coiled. Spermiogenesis in the whip scorpion starts out in a manner basically similar to spermiogenesis in species with conventional elongate sperm. As in many other animal species, the spermatid nucleus is surrounded by a manchette of microtubules and elongates in an axis parallel to the microtubules. Late in development, however, the nucleus and flagellum undergo unorthodox contortions in which the nucleus becomes spiralized and the flagellum is wound up into the body of the cell. No microtubules appear to be associated with the spermatid nucleus during the process of coiling. Pronounced changes in the configuration of chromatin occurs concomitantly with the coiling which suggests that chromatin alteration may be related to the changes in nuclear shape. Peculiar spermatozoa similar to the type found in Mastigoproctus have been described in spiders, whereas scorpions have more conventional sperm. Thus, whip scorpions may be more closely related to spiders than scorpions.

The whip scorpions (Uropygi) are found throughout tropical and semitropical parts of the world. They are often called vinegarones because of an acetic acid containing fluid which they secrete when they are irritated. The Uropygi are scorpionlike in general appearance (Fig. 1), and some textbooks consider them to be closely related to scorpions (6). Recently, however, it has been suggested that the whip scorpions may be more closely related to spiders than scorpions (14). It has been suggested that sperm morphology could be used as a phylitic trait for analysis of evolutionary relationships (3, 1). Early events during spermiogenesis, in Mastigoproctus appear very similar to early spermiogenesis in a broad range of animals. However, late in sperm development in Mastigoproctus, the nucleus undergoes pronounced and unusual changes. The only other known group with similar sperm are the spiders (13, 12). The similarity of spermiogenesis in Mastigoproctus to spermiogenesis in spiders suggest that whip scorpions may be more closely related to spiders than scorpions. Thus, this case of a really unusual type of spermiogenesis may help to decipher a difficult classification problem.

In addition, spermiogenesis in the whip scorpion provides an example of profound changes in nuclear shape occurring in the absence of microtubules.

MATERIALS AND METHODS

Mastigoproctus giganteus were caught in Southern Arizona by Mr. David Resnick who kindly gave them to me. Testes were fixed in 4% collidine-buffered glutaraldehyde, postfixed in collidine buffered 1% OsO₄, dehydrated in alcohol, and embedded in Epon. Sections were stained in 3% aqueous uranylacetate and lead citrate.

RESULTS

As in many other invertebrates which have been described (11), spermiogenesis in Mastigoproctus occurs in cysts. Each cyst consists of a group of germ cells surrounded by an epithelium. Within a cyst germ cells are interconnected by cytoplasmic bridges and develop nearly synchronously.

In early spermatids the nucleus is roughly spherical. Two centrioles, which are located in an indentation in the nucleus, are so aligned that a straight line could
pass through the center of both of them (Fig. 2). This is somewhat unusual as the two centrioles of a pair are generally situated at right angles to one another. The centriole furthest from the nucleus will serve as a basal body for the forming spermatid flagellum. The centriole nearest the nucleus will serve as a basal body for the forming spermatid flagellum. The centriole nearest the nucleus disappears during early spermio genesis and striated material appears in its place. A small dense ring or annulus, about 0.5 μm in diameter, is situated immediately posterior to the basal body. The annulus appears to be attached to the plasma membrane which is involuted around the flagellum in very early stages of development (Fig. 2). A concentration of ribosomes occurs adjacent to the flagellum.

As spermiogenesis proceeds, the formerly spherical nucleus begins to elongate (Fig. 3). Chromatin appears as thick (200 Å) strands. The chromatin filaments, particularly those located more medially, become disposed roughly parallel to the long axis of the nucleus. The nuclear membrane does not follow the contours of the condensing chromatin; rather, it appears to be loosely fitting, with a space of as much as 1 μm between the dense chromatin and the nuclear membrane (Fig. 3). When the nucleus has become about two or three times as long as it is wide, it extends from one side of the cell to the other. Annulli appear to be located at the posterior of the nucleus only. Developing acrosome and flagellum extend from opposite sides of the spermatid nucleus.

As the nucleus continues to elongate the whole spermatid also elongates to achieve an overall oval profile. Chromatin fila-
Fig. 2. Young spermatid with nearly spherical nucleus: Annulus, an; cytoplasmic bridge, b; proximal centriole, p; distal centriole, d. × 29 000.

Fig. 3. Later stage than in Fig. 2. Chromatin has condensed into cylindrical shape in the center of the nucleus. Chromatin filaments are arranged parallel to the long axis of the nucleus. Acrosome; a. × 21 000.
ments become increasingly more regularly disposed. They appear to be disposed roughly parallel to the long axis of the nucleus. The cigar-shaped nucleus develops a bend in the center (Fig. 4). The nuclear membrane remains loosely wrapped with an electron lucid region around more solid-appearing central chromatin mass. When the nucleus is viewed in transverse section, the 200 Å chromatin filaments appear in cross section in the center of the nucleus and oblique near the periphery of the chromatin mass (Fig. 5). This implies that the more peripheral chromatin filaments may be twisted around the central filaments. A manchette of about 100 evenly spaced microtubules is situated just peripheral to the nuclear membrane. When the nucleus is viewed in transverse section, the microtubules on one side of the nucleus appear in transverse section whereas those microtubules on the opposite side are cut obliquely (Fig. 5). This implies that the microtubules are disposed generally parallel to the long axis of the nucleus but that the manchette may be somewhat irregular or twisted.

As the nucleus continues to elongate it takes on the form of a gentle S. Some sections which transect the acrosome and the flagellum also cut through the nucleus along its entire length, indicating that the curved places are roughly two-dimensional (Fig. 6). Chromatin in the center of the nucleus, distal to the acrosome and flagellar base, appear somewhat more electron dense than the chromatin at the anterior and posterior regions of the nucleus. The cytoplasm is concentrated at the posterior half of the nucleus (Fig. 6).

Pronounced changes occur late in spermiogenesis as the long thin spermatid is transformed into a spherical cell. These alterations include (1) change in chromatin structure from parallel 200 Å filaments to loosely aggregated 50 Å filaments; (2) movement of the manchette microtubules from just outside the nuclear membrane to just beneath the plasma membrane and subsequent disappearance of the microtubules; (3) coiling and twisting of the nucleus into a tight spiral; and (4) coiling of the flagellum into the cell body.

Movement of microtubules apparently occurs rapidly since microtubules between the nucleus and plasma membrane are rarely observed. In sections where microtubules are observed midway between the nucleus and plasma membrane, they generally occur in short rows (Fig. 8). Transformation of chromatin structure from ordered 200 Å filaments to clumps of 50 Å
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FIG. 5. Transverse section of two spermatid nuclei. Chromatin is arranged in thick filaments. One has the impression from such images that the chromatin filaments may be twisted. Microtubules are juxtaposed to the nuclear membrane. × 39 000.

filaments also apparently occurs rapidly as nuclei with both types of arrangements are sometimes observed in the same cyst (Fig. 8). As chromatin is rearranged, the nucleus begins to coil (Fig. 7). The entire nucleus comes to be situated inside of a roughly spherical cell as a U shape (Fig. 9). In later stages, the nucleus is observed to be wound into a very tight coil (Fig. 10). In very late spermatids, concomitant with the coiling of the nucleus, the flagellum is drawn into the cell where it is observed in about eight concentric coils devoid of plasma membrane (Fig. 10). The flagellum of Mastigoproctus spermatids, like spider sperm flagella, has a 9 + 3 tubule pattern rather than the typical 9 + 2 pattern. The profiles of portions of the flagellum posterior to the termination of the central tubules are found together as would be expected if the flagellum were coiled up regularly like a spring.

DISCUSSION

Although manchette microtubules have been implicated in nuclear shaping during spermiogenesis in some animals (7, 8, 16), in other species shaping of the spermatid nucleus may be effected by ordered compacting of chromatin (10, 9, 2, 15). This has raised a general controversy about nuclear shaping during spermiogenesis which has recently been discussed in detail. For a discussion of the evidence for microtubules as effectors of nuclear shaping, see (16), and for discussion in support of chromatin condensation as the effector of nuclear shape, see (2). In Mastigoproctus, the spermatid nucleus undergoes a dynamic change in shape late in spermiogenesis. Microtubules do not appear to play a major role in this change in shape since the manchette microtubules have moved outward and away from the nucleus before the shape change occurs, and they disappear considerably before the final shape of the nucleus is achieved. At the time when the nucleus begins coiling, ordered 200 Å chromatin filaments become rearranged into 50 Å filaments. The temporal correla-
Fig. 6. Later stage than Fig. 4. The shaping spermatid nucleus has the form of a gentle S. × 15,000.

Fig. 7. Later stage than Fig. 6. The nucleus becomes a plainar as it takes a coiled conformation. The chromatin filaments have taken on a different appearance; they appear thinner and aggregated. × 17,000.

tions suggest that changes in chromatin structure could bring about gross changes in nuclear shape.

During early stages of spermatal development a manchette is present adjacent to the nuclear membrane. However, the nuclear membrane is very loosely filled to the regularly shaped chromatin mass during
these early stages. It is difficult to see how force generated by the manchette could bring about a complex and precise change in shape of the chromatin mass from across the distance of the intranuclear space.

The classification of whip scorpions (Uropygi) is uncertain. They have been considered to be closely related to scorpions (6). This classification, however, has been questioned (4), and Savory has suggested that Uropygi may be more closely related to spiders than scorpions (14). Our observations support Savory’s classification as spermiogenesis in *Mastigoproctus* is strikingly similar to the unusual type of sperm development which occurs in spiders and dissimilar to sperm development in scorpions. Spider spermatids undergo similar coiling of nucleus and flagellum.
Fig. 9. Two spermatids in stage where nucleus and flagellum are coiling. Chromatin has changed in appearance. Chromatin filaments are thin and in clumps and appear twisted. × 25 000.

Fig. 10. Later stage than Fig. 9. Nucleus and flagellum have become coiled within spherical spermatid. The three central tubules apparently extend only about half the length of the flagellum as they are only seen in half the profiles of transversely sectional axoneme. The axonemes with central tubules are situated together suggesting that the flagellum is coiled in a regular fashion. × 42 000.
into a spherical spermatozoan (13, 12), and they also possess an atypical 9 + 3 flagellum. Scorpions, on the other hand, produce a long slender spermatozoan and have a typical 9 + 2 flagellar tubule pattern (10, 5).

REFERENCES