NUCLEAR SHAPING IN THE ABSENCE OF MICROTUBULES IN SCORPION SPERMATIDS

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INTRODUCTION

During spermiogenesis in most animal species, sperm nuclei undergo morphogenesis which results in the transformation of relatively large spherical nuclei with diffuse chromatin into small elongate or flattened nuclei with highly condensed chromatin. The shapes of nuclei of mature spermatozoa are beautifully precise and species-specific.

There is no general agreement among cell biologists as to the forces which effect nuclear shaping. Some investigators have suggested that microtubules which surround spermatid nuclei during shaping play an active role in this process (McIntosh and Porter, 1967; Kessel, 1966, 1970), whereas others have suggested that nuclear condensation is effected by internal condensation of chromatin (Fawcett et al., 1971). It has also been suggested that nuclear condensation and shaping could result from the interplay of nucleus, cytoplasmic organelles, and supportive cells (Phillips, 1970; Rattner, 1972). In this report, I shall describe nuclear shaping during spermiogenesis in two species of scorpion where the formation of a highly compacted sperm nucleus from a large spherical nucleus appears to be effected without interaction with microtubules.

MATERIALS AND METHODS

Testes of the scorpions Hadrurus hirsutus and Veiuous spinigerus were fixed at room temperature in 0.2 M Sorenson's phosphate-buffered 4% glutaraldehyde containing 0.1% CaCl₂ and 0.1% MgCl₂. Testes fixed for 1-4 h were postfixed in 1% collidine-buffered OsO₄ for 1-2 h, dehydrated in alcohol, embedded in Epon, and stained in uranylacetate and lead citrate.

RESULTS

The ultrastructure of nuclear shaping during spermiogenesis in H. hirsutus is very similar to that in V. spinigerus. I shall, therefore, describe them together. The scorpion testis is divided into cysts. Cysts contain either spermatocytes or spermatids. Those cysts which have spermatids contain approximately 1024 (2¹⁰) cells at nearly the same stage of development. Spermatids are not in direct contact with the cyst wall, nor are they embedded in any type of supportive cell (Jespersen and Hartwick, 1973).

The nucleus of young spermatids is spherical. Chromatin is more condensed in the region of the nucleus nearest the base of the flagellum (Fig. 1). No microtubules are observed in association with the nucleus. As spermiogenesis continues, the nucleus gradually takes on a more regular and elongate shape and the chromatin becomes gradually more compacted (Figs. 2-7). Chromatin filaments become aligned parallel to the long axis of the nucleus (Figs. 3 and 5). Chromatin filaments appear to fuse into lamellae in later stages (Fig. 6). When nuclear condensation is complete, the long, pencil-shaped nucleus contains highly condensed, homogeneously electron-dense chromatin (Fig. 7). No microtubules are found near the spermatid nuclei during any stage of development (Figs. 1-7). The developing acrosome is small and is associated only with the anterior end of the spermatid nucleus (Figs. 2 and 3).
Although there are no microtubules associated with spermatid nuclei in *Hadrurus* and *Vejous*, microtubules are readily observed in spermatogonia (Fig. 8) and spermatocytes (Fig. 9) fixed in the same way. This demonstrates that microtubules are being preserved in this material by our fixation procedure.

DISCUSSION

Microtubules appear not to be involved in nuclear shaping and condensation in *H. hirsutus* or *V. spinigerus* since none are observed around the nucleus. It is highly unlikely that microtubules existed around the nucleus but were not preserved in preparation, as some microtubules encircling spermatid nuclei are particularly stable. This is evident from the observation that they are preserved by OsO₄ fixation (Burgos and Fawcett, 1955). The fixation used in our experiments (phosphate-buffered glutaraldehyde at room temperature with divalent cations) is known to preserve microtubules in a wide variety of tissues. The
fixation was apparently satisfactory in the scorpion testis in this instance since numerous well-preserved microtubules were observed in spermatogonia and spermatocytes in the same material. Supportive cells cannot be involved in nuclear shaping in spermatids of these scorpion species since spermatids develop free in the cyst lumen without associations with supportive cells or with each other. It is also unlikely that the acrosome is involved in nuclear shaping as the acrosome in both species is small and located only over the anterior-most end of the spermatid nucleus. The
regular arrangement of chromatin during spermiogenesis suggests that precise packing of chromatin could effect nuclear shaping.

The results of this report show that in two species of scorpion, nuclear condensation and shaping are apparently caused without interaction between cytoplasmic microtubules and nuclei. It may be that in other species, nuclear shaping is brought about by interactions with microtubules. There is good evidence implicating microtubules in nuclear shaping in the chicken (McIntosh and Porter, 1967). The scorpion situation does indicate, however, that a typical, highly-condensed, long, thin sperm nucleus can be formed without interaction with microtubules.

**SUMMARY**

During sperm development in the scorpions *H. hirsutus* and *V. spinigerus*, the nucleus becomes elongate and condensed. Though microtubules appear around the spermatid nucleus during spermiogenesis in other animal species, nuclear elongation in the scorpion occurs in the apparent absence of microtubules. Neither spermatid nucleus nor
acrosome in these species is associated with supportive cells which could influence their shaping. It is suggested that chromatin condensation could determine nuclear shape.

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