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Details of Sperm Tail Fibrous Sheath Revealed by Chemical Dissection and Unembedded Electron Microscopy

Maya Markova

Department of Biology, Medical Faculty, Medical University of Sofia, Sofia, Bulgaria

*Corresponding author e-mail: mayamarkov@gmail.com

The fibrous sheath is a cytoskeletal structure in the sperm tail principal piece composed of two longitudinal columns and multiple transverse ribs. To study details of its structure, we subjected mouse and human spermatozoa to extraction for fibrous sheath, a chemical dissection procedure which removes all other tail components. Extracted fibrous sheaths were observed by unembedded whole-mount transmission electron microscopy. In the distal part, the ribs tended to show a paired arrangement. It apparently reflected the paired structure of the anlagen during spermiogenesis, though this peculiarity was thought to be lost in the mature spermatozoon. Our data suggest that the original paired spines persist in a hidden form in the definitive ribs. Another observation was an apparent widening of distal fibrous sheath up to two times. We suppose that the spacial arrangement of ribs during spermiogenesis is achieved by intercalation, and the process is reversed by the chemical dissection.

Key words: Sperm tail, fibrous sheath, cytoskeleton, whole-mount, ultrastructure

Introduction

The fibrous sheath is a periaxonemal cytoskeletal structure specific for the sperm tail principal piece of therian mammals. It overlies the axoneme and outer dense fibers and consists of two longitudinal columns associated with microtubular doublets 3 and 8, and numerous transverse semicircular ribs interconnecting them. Despite having no motor activity of its own, the fibrous sheath contributes to sperm progressive motility by influencing tail flexibility [4]. It also serves as an attachment scaffold for various proteins, such as glycolytic enzymes [3]. Spermatozoa of some infertile men show fibrous sheath defects, either with a specific phenotype called dysplasia of the fibrous sheath, or in a broader category known as non-specific flagellar anomalies [2]. These defects are associated with loss of motility that may require treatment by

intracytoplasmic sperm injection (ICSI) [1]. At least in some cases, fibrous sheath abnormalities are the root cause of infertility, as shown in patients and mouse models with mutations affecting its components [10]. Because of this functional importance, numerous studies on fibrous sheath structure and biogenesis have been carried out. It has been reported to assemble in distal-to-proximal direction during spermiogenesis, first the angagen of the longitudinal columns and then the rib anlagen. The latter is initially a series of evenly spaced double spines which then attach to the longitudinal columns, thicken, group and coalesce by deposition of additional electron-dense material to form the mature ribs. The deposition and coalescence is more pronounced in the proximal principal piece [5]. The complexity and high electron density of the definitive fibrous sheath poses challenges to investigations of its structure. We used chemical dissection and whole-mount electron microscopy of mouse and human fibrous sheath to find details that are not revealed by conventional protocols.

Materials and Methods

Mouse spermatozoa were obtained from the vas deferens and cauda epididymidis of adult Swiss males, and human ejaculates were donated by healthy normozoospermic volunteers. The study conformed to EU and Bulgarian legislature and ethical guidelines for research.

The sperm cells were extracted for fibrous sheath according to Kim et al. [6, 7]. After washing with phosphate-buffered saline (PBS), pH 7.2, with 0.2 mM phenylmethylsulfonyl fluoride (PMSF), they were incubated twice for 15 min at 4°C with shaking in 50 mM Tris-Cl (pH 8.8) with 2% Triton X-100 and 5 mM dithiothreitol (DTT). Then the spermatozoa were washed in 50 mM Tris-Cl (pH 8.8) with 0.2 mM PMSF. After that, they were incubated for 5 hours at 4°C with shaking in 25 mM Tris-Cl (pH 7.5) with 4.5 M urea and 25 mM DTT, followed by washing first in 25 mM Tris-Cl (pH 7.5) and then in PBS (pH 7.2).

The extracted sperm cell remnants were spread on formvar-coated grids, fixed with 2.5% glutaraldehyde in PBS for 30 min at 4°C, washed with PBS and postfixed with 1% OsO_4 in PBS for 5 min at 4°C. Then they were washed twice with water for 5 minutes each, stained with 1% or 2% uranyl acetate and observed by a transmission electron microscope.

Results and Discussion

Whole-mount electron microscopy after removal of most sperm tail components by chemical dissection allowed us to observe the fibrous sheath in its entirety and to trace structural details. The applied extraction procedure produced similar results in human and mouse spermatozoa, leaving the fibrous sheath as the only visibly preserved tail structure (**Fig. 1**). While the thick and robust proximal fibrous sheath looked largely intact, damage to the longitudinal columns and stretching of the ribs was commonly observed in the distal fibrous sheath.

More details could be observed in distal than in proximal fibrous sheath regions, due to the lower electron density. Ribs tended to show a paired arrangement. These

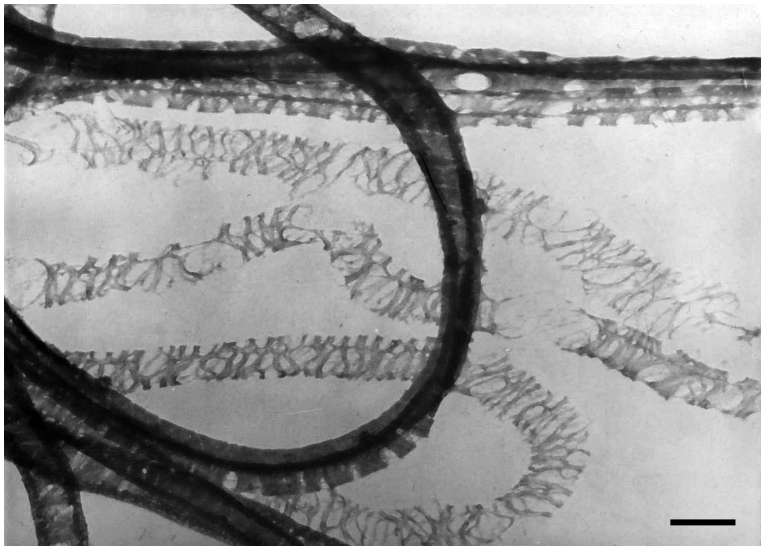

Fig. 1. A representative view of a human fibrous sheath preparation. Bar = 500 nm.

pairs, visible in some of the tail remnants in **Fig. 1**, were most apparent in fibrous sheaths that had been stretched during the processing (**Fig. 2**).

Towards the distal end of the fibrous sheath, its apparent width often increased up to two times. The easiest explanation was two-dimensional spreading after disintegration of one of the longitudinal columns. This could be the case for some fibrous sheaths

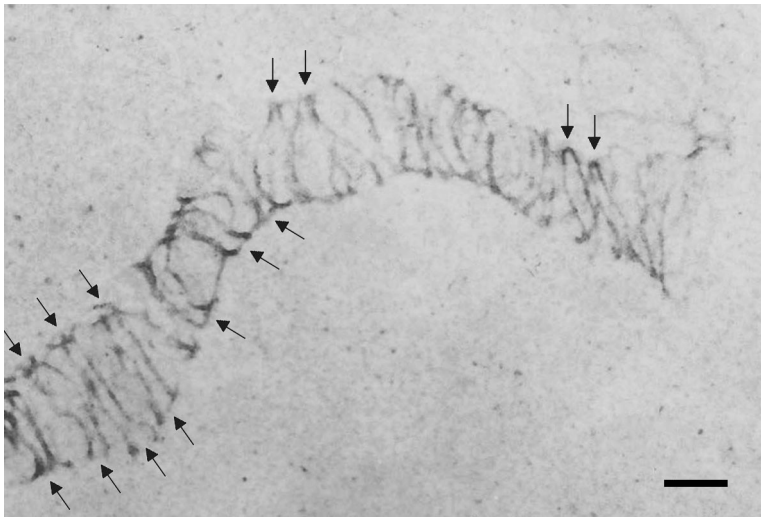

Fig. 2. Streched, visibly damaged mouse distal fibrous sheath. Ribs are apparently arranged in pairs, some of which are indicated by arrows. Bar = 500 nm.

such as the one in **Fig. 2**, but detailed view of others suggested pulling apart of ribs still attached with one of their ends to opposite longitudinal columns (**Fig. 3**).

We have previously observed interesting ultrastructural details in mouse and human sperm cells by whole-mount electron microscopy after another type of chemical dissection – extraction for nuclear matrix and intermediate filaments [8, 9]. Now, we applied a similar approach specifically to the sperm tail fibrous sheath, a tissue-specific cytoskeletal component that is still poorly understood because of inherent technical difficulties associated with its study. Chemical dissection facilitates observation by removing fractions of the cellular content to expose the structure of interest, and whole-mount unembedded electron microscopy circumvents the difficulties in interpreting images of a three-dimensional structure on ultrathin sections.

Extraction for fibrous sheath uses non-ionic detergent, DTT and urea to dissolve all other sperm tail components [6, 7]. Our observations showed that ribs of extracted fibrous sheaths tended to occur in pairs. This apparently corresponded to the paired anlagen described in [5] during spermiogenesis, but we have not found in the literature observations of paired fibrous sheath ribs in mature spermatozoa. We could hypothesize that the original double spines persist in the definitive ribs and are only masked by overlaying material that is partly removed or relaxed by the chemical dissection and mechanical stress applied during the preparation for microscopy. Another interesting observation was the apparent widening of distal fibrous sheath, even when both longitudinal columns still had ribs attached to them. We suppose that the spacial arrangement of ribs during spermiogenesis is achieved by intercalation, and the process is partly reversed by the chemical dissection.

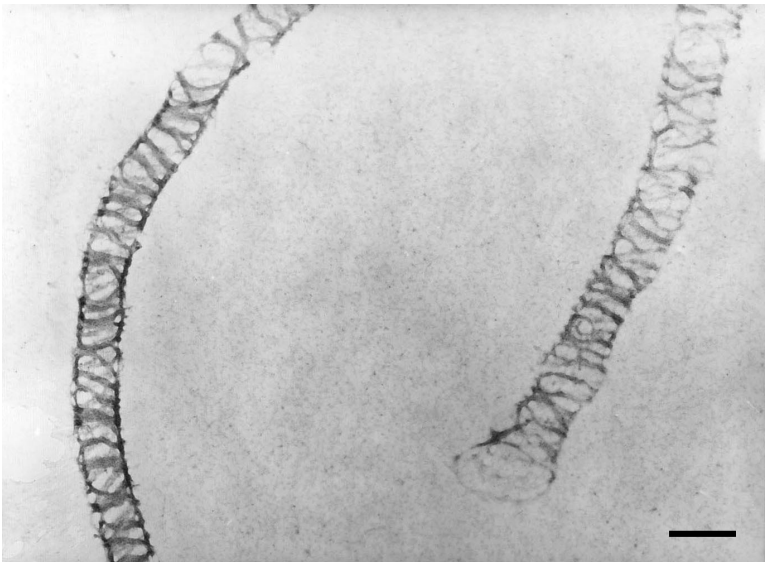

Fig. 3. Two regions of the fibrous sheath of the same mouse sperm tail. At the distal end (bottom right), the diameter increases twice, and ribs attached to opposite columns are seen pulled apart. Bar = 500 nm.

Conclusions

Our observations of sperm tail whole-mounts after extraction for fibrous sheath show that the paired structure of the rib anlagen persists in a hidden form in the definitive ribs, and suggest that their positioning during fibrous sheath formation is a result of intercalation.

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