The spermatozeugmata of *Tubifex tubifex* (Annelida, Clitellata, Tubificinae) studied by different technical approaches

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Keywords: structure, spermatozoa, actin, dichotomous sperm lines, flagella

Oligochaete annelids belonging to the subfamilies Tubificinae and Limnodriloidinae are characterized, *inter alia*, by the production of two different sperm types, named eusperm and parasperm [1]. In *Tubifex tubifex* the two sperm types differ in nearly all their characters, like nuclear size [(about 30 μm in the eusperm, but 3 μm in the parasperm); the amount of nuclear DNA (about eight times more in the eusperm than in the parasperm); the extreme reduction of the acrosome in the parasperm; the size of the mitochondria (about double in the parasperm with respect to the eusperm); and the tail (the cell membrane is separated from the axoneme by a consistent space in the parasperm, and the space is filled by γ glycogen, whereas in the eusperm the space is reduced and has β glycogen granules)]. We have studied the production of the two sperm types, and we found that, starting from similar spermatocytes, euspermatooza are produced through a regular meiotic process, whereas the paraspermatozoa are produced through a peculiar process of nuclear fragmentation which, produces a very high number of aneuploid parasperm [2].

When fully mature, the two types of sperm are transmitted to the partner during copulation, and both reach the spermathecae. There, the two sperm types are grouped to form typical bundles, called spermatozeugmata, rod-shaped structures, up to 2 mm long, already known since 1861 [3]. The spermatozeugmata have an outer layer, the cortex, formed by tightly packed parasperm, and an inner core, the axial cylinder, formed by paralelly arranged eusperm. The spermatozeugmata are stored in the spermathecae up to the moment of fertilization, when they move towards the spermathecal duct at the edge of which they are broken, thus delivering the eusperm [3]. Thus, the spermatozeugmata perform different functions, accompanied by particular features:

- they hold together a certain number of fertilizing sperm "ready for use". This is probably accomplished by an impressive complex of cell junctions formed mainly by septate junctions, followed by scalariform junctions connecting parasperm tails [3]
- they are able to move with a metachronal wave formed by the distal extremities of the parasperm, thus carrying the fertilizing eusperm [3].

We have studied *T. tubifex* spermatozeugmata using different techniques: TEM, SEM, freeze fracture, high speed video microscopy, confocal microscopy and immunogold labeling.

We were able to show the metachronal wave produced by the parasperm tails and measure its beat frequency (10 Herz). For the first time using CLSM we have documented by a TRITC-phalloidinlabeling the presence of a large amount of F-actin inside the cytoplasm of the parasperm tails. Actin was also localized by means of immunogold staining under TEM. It remains to be established to what extent actin is connected to the septate junctions.

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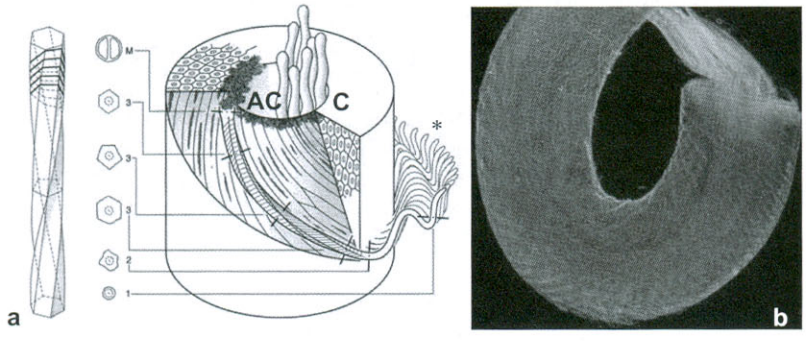

Figure 1. (**a**) A highly schematic drawing showing the spermatozeugma architecture: the fertilizing eusperm are parallely arranged in the central, axial cylinder (AC), and surrounded by the parasperm connected by a long series of junctions in the cortex (C). Only the parasperm extremities are free to move and form a metachronal wave (asterix). To the left, a single, enlarged parasperm tail shows the oblique arrangement of the junctions with respect to the tail's axis. (**b**) A CLSM image of a broken spermatozeugma releasing the eusperm from the axial cylinder, showing the DNA of the eusperm heads (blue), the tubulin of eusperm and parasperm tails (green) and F-actin (orange) localized in the parasperm tails.

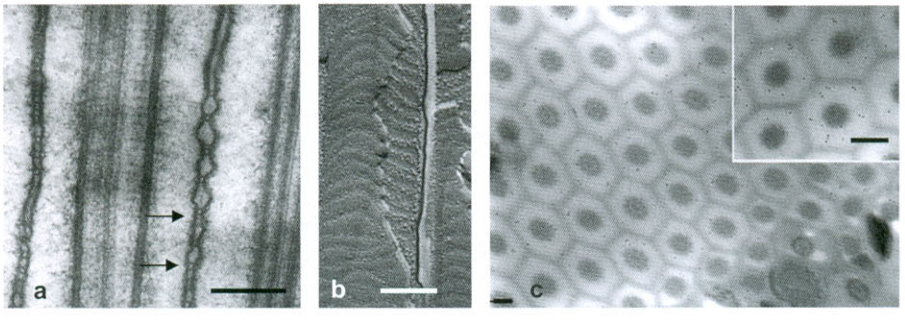

Figure 2. (**a**) Longitudinal section of two parasperm flagella. Note the wide space between axoneme and plasma membrane and the junctions connecting two neighbouring tails (arrows). (**b**) A freeze-fracture view of a field similar to that in **a** to show the alignment of particles forming cell junctions. (**c**) Immunogold labeling of actin inside the parasperm tails grouped in the cortex, to show the localization of actin in the periaxonemal space. Bars are 0.2 μm