Available online at www.sciencedirect.com
SCIENCE DIRECT
Zoologischer Anzeiger 245 (2006) 3–12
ZOOLOGISCHER ANZEIGER
www.elsevier.de/jcz

Ultrastructure of spermiogenesis and spermatozoa in *Mesocastrada fuhrmanni* Voltz, 1898 (Platyhelminthes, Rhabdocoela, Typhloplanoida)

Julia-Laurence Culioli*, Joséphine Foata, Christophe Mori, Antoine Orsini, Bernard Marchand

Laboratoire Modèles en Ecologie Méditerranéenne, Faculté des Sciences et Techniques, Université de Corse, F-20250 Corte, France

Received 31 January 2006; received in revised form 28 February 2006; accepted 2 March 2006
Corresponding editor: M.V. Sørensen

Abstract

Electron microscopy of the testes of the free-living flatworm *Mesocastrada fuhrmanni* collected from temporary freshwater ponds shows stages of spermiogenesis that are like other species of the Typhloplanidae. Spermiogenesis in *Mesocastrada fuhrmanni* is characterized by the presence, in the spermatid, of a differentiation zone underlain by peripheral microtubules and centered on two centrioles with an intercentriolar body. Two flagella of the 9+"1" pattern of the Trepaxonemata grow out in opposite directions from the centrioles. The flagella undergo a latero-ventral rotation, and a subsequent disto-proximal rotation of centrioles occurs in the spermatid. The former rotation involves the compression and the detachment of a row of cortical microtubules, and allows us to recognize a ventral from a dorsal side. Two features are of special interest at the end of differentiation: peripheral cortical microtubules lie parallel to the sperm axis near the anterior tip, but microtubules become twisted (about 40° with reference to the gamete axis) near the posterior extremity; in the same way, the posterior tip of the nucleus is spiralled. As far as we know, these features are observed for the first time in the Typhloplanidae. The pattern of spermiogenesis and the ultrastructural organization of the spermatozoon are compared with the available data on Typhloplanoida and in particular, species of the Typhloplanidae family.

Keywords: Spermiogenesis; Spermatozoon; Ultrastructure; *Mesocastrada fuhrmanni*; Typhloplanoida

1. Introduction

The Rhabdocoela constitute a heterogeneous taxon. Typhloplanidae are mostly aquatic (marine and freshwater) platyhelminths belonging to the order Typhloplanoida.

Over the past, studies dealing with the Rhabdocoela sperm ultrastructural characteristics have been realised (Hendelberg 1969a, b, 1977; Rohde 1987; Cifrian et al. 1988a, b). The evolutionary significance of these ultrastructural features has been particularly investigated by Ehlers (1984, 1985a, b, 1986), Hendelberg (1986), Watson (1999, 2001), Watson and Rohde (1995), Joffe and Kornakova (1998).

A complete description of ultrastructural characteristics of spermiogenesis and spermatozoa in the Typhloplanidae seem necessary to evaluate the differences and the relationships between representatives of this family and the members of the other Typhloplanoida

*Corresponding author. Fax: +33 4 95 45 00 29.
E-mail address: jculioli@univ-corse.fr (J.-L. Culioli).

0044-5231/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jcz.2006.03.002

families. Therefore, we discuss in this paper the ultrastructural organization of spermiogenesis and the mature spermatozoon in *Mesocastrada fuhrmanni* Voltz, 1898 (Typhloplanoida, Typhloplanidae), a flatworm found in temporary ponds of Corsica. Recently, epidermis and protonephridia of *Mesocastrada fuhrmanni* have been described (Culioli 2006).

2. Material and methods

Live specimens of *Mesocastrada fuhrmanni*, were collected in several temporary ponds (Padulellu, Padulu) of Corsica Island (France). The species are free-living and move at the surface of the water. Adult specimens were fixed in cold (4 °C) 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2 for 2 h, rinsed in 0.1 M sodium cacodylate buffer at pH 7.2, postfixed in cold (4 °C) 1% osmium tetroxide in the same buffer for 1 h, rinsed in 0.1 M sodium cacodylate buffer at pH 7.2, dehydrated in ethanol solutions and propylene oxide, embedded in Epon and polymerized at 60 °C for 48 h. Ultrathin sections (60–90 nm) of several specimens were obtained using a LKB ultrotome III, placed on 300 mesh copper grids and double-stained with uranyl acetate and lead citrate following Reynolds (1963) methodology. The grids were examined under an Hitachi H-600 electron microscope at 75 kV in Corsica University.

3. Results

3.1. Spermiogenesis

The two testes of *Mesocastrada fuhrmanni* contain all the stages of spermiogenesis. Primary spermatocytes (Fig. 1A), mostly located at the periphery of the testes, are large cells recognizable by several synaptonemal complexes (Fig. 1B) in an irregular-shaped nucleus. Clusters of spermatids contain mitochondria and a well-developed Golgi apparatus giving rise to several dense bodies. These latter are heterogeneous structures including a translucent component with a dense core. Spermatids are characterized by huge vacuoles from the early stages of spermiogenesis (Fig. 1C–D). At the beginning of differentiation, the nucleus is spherical and has a scattered chromatin (Fig. 1C and D).

Spermiogenesis starts in very young cells by the formation of a zone of differentiation (ZD) marked by arched membranes (Figs. 1C, D and 3A). By that time, cells have not realized their last cytoplasmic division yet (Fig. 1C). The ZD include two rows of microtubules underlined by dense material (Fig. 1C), and contains two centrioles made up of triplets (Fig. 1F) with an intercentriolar body (Ib) in between. Two flagellae of the 9 + "1" Trepaxonemata pattern grow out in opposite directions (Fig. 3B). Each centriole is linked to both the Ib and the nucleus by short "striated rootlets" (Fig. 1F). Ib appears in young spermatids (Fig. 1C) and consists of a dark central ribbon flanked by two dark lateral ribbons and separated by two electon-lucent ones in which fine cross filaments can be distinguished radiating from the central element toward the side elements (Fig. 2A). During spermiogenesis, both the Ib and the flagella undergo transformations. Flagella start a 90° rotation until becoming parallel to each other and perpendicular to the cell axis (Figs. 2A, B and 3C). Spermatids have not undergone their last cytoplasmic division at this stage. This latero-ventral rotation is responsible for a dissymmetry of the ZD and differentiates a ventral face (flagellar) and a dorsal face (aflagellar) in the spermatid. The latero-ventral rotation induces the compression of one row of peripheral cortical microtubules (ventral) which become enclosed into the ZD (Figs. 2D, E and 3C). During the rotation, the central element of the Ib stretches and the side ones diverge (Figs. 2C and 3C, D). The side elements of the Ib transform into a transitory electron-dense structure still joined with centrioles (Fig. 2C) before becoming a spur-shaped process which remains in the mature spermatozoon (Figs. 2D and 3D). A distal projection, corresponding to the future anterior end of the spermatozoon appears in the spermatid and the spermatid elongates (Figs. 1C, 2H and 3C). Centrioles begin a disto-proximal rotation until becoming parallel to the cell axis (Figs. 2E and 3D). During the elongation, the nucleus, in which chromatin takes on a honeycomb-like pattern, stretches (Figs. 2F and G). At the end of elongation, the peripheral cortical microtubules remain partially twisted. The nucleus also becomes spiralled. No relative movement between centrioles and nucleus (still linked by striated rootlets) was observed. At the end of spermiogenesis, striated rootlets and Ib disappear. A long and filiform late spermatid with two free flagella of the Trepaxonemata 9 + "1" pattern anchored near the anterior extremity and with both twisted microtubules and nucleus is the product of this differentiation.

3.2. Spermatozoon

The spermatozoon of *Mesocastrada fuhrmanni*, observed under light microscopy undergoes random sinusoidal undulating movements, making it difficult to distinguish the anterior tip from the posterior one.

We have studied mature spermatozoa in the seminal vesicle and sperm ducts by TEM.

The spermatozoon of *Mesocastrada fuhrmanni* is filiform in shape, tapered at both ends, lacks an acrosome, and is equipped with two sub-equal flagellae