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Sperm nucleomorphogenesis in the cephalopod *Sepia officinalis*

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Abstract

Sperm nucleomorphogenesis in the cephalopod *Sepia officinalis* is the product of the interaction between perinuclear microtubules and condensing chromatin. This interaction occurs during spermiogenesis and is established through the nuclear membrane.

As in other cephalopod species, the perinuclear microtubules are transient structures. In the case of *S. officinalis*, they begin to appear in the basal area of the early spermatid and progress from there, establishing contact with the external nuclear membrane and follow a defined, but not symmetric, geometry. Thus, the microtubules accumulate preferentially in one area of the nuclear membrane which we refer to here as the "dorsal zone". Later, the microtubules will be eliminated before the mature spermatid migrates to the epididymis.

The chromatin is condensed within the nucleus following a complex pattern, beginning as fibro-granular structures until forming fibres of approximately 45 nm diameter (patterning phases). From this stage on, an increase in the chemical basicity of DNA-interacting proteins is produced, and chromatin fibres coalesce together, being recruited to the dorsal zone of the membrane, where there is a higher density of microtubules. This last step (condensation phases) allows the chromatin fibres to be arranged parallel to the axis of the elongating nucleus, and more importantly, is deduced to cause a lateral compression of the nucleus. This lateral compression is in fact a recruitment of the ventral zone toward the dorsal zone, which brings about an important reduction in nuclear volume.

The detailed observations which comprise this work complement previous studies of spermiogenesis of *Sepia* and other cephalopods, and will help to better understand the process of cellular morphology implicated in the evolution of sperm nuclear shape in this taxonomic group.

Keywords: Cephalopods; *Sepia officinalis*; Spermiogenesis; Nucleomorphogenesis; Chromatin; Microtubules

1. Introduction

The various levels of biological organization related to the reproduction of species have been exposed to an exceptionally fast evolution. This applies at the molecular level (Wyckoff et al., 2000; Swanson and Vacquier, 2002; Lewis et al., 2004), at the cellular level (Jamieson et al., 1995), as well as to the great variety of the reproductive organs and types of fertilization that organisms display.

A particular case on which we have been working is based on the evolutionary changes of sperm nuclear shape of cephalopods, as well as the proteins that condense spermiogenic and spermatic chromatin (Giménez-Bonafé et al., 2002a, 2002b, 2004; Ribes et al., 2004; Martínez-Soler et al., 2007). From studies of conventional optical microscopy, Franzén (1967) defined the evolutionary trend that has occurred in sperm of the phylogenetic group including cephalopod decapods and the octopi *Octopus* and *Eledone*. This trend consists of a nuclear elongation followed by a remarkable spiralization of the nucleus. It is important to mention that this evolution is accompanied by changes in other parts of the cell, mainly in the acrosome and axoneme, which are not taken into account in this work. The nuclear elongation is first produced during the evolutionary divergence: [*decapods-Octopus*], as well as an exclusive spiralization of the acrosome (Galangau and Tuzet, 1968; Longo and Anderson, 1970; Ribes et al., 2002). Later, in some octopi such as *Eledone*, nuclear elongation becomes

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much more severe, and the whole sperm nucleus is spiralized along its elongation axis (Maxwell, 1974; Giménez-Bonafé et al., 2002a).

Despite the fact that the study of variation of shape and components of the mature sperm cell has been extensively used for studies of taxonomy and phylogenetics (see for instance Franzén, 1977; Jamieson, 1991; Jamieson et al., 1999), the studies on the evolutionary changes of the spermiogenic processes are scarcer. In the previously cited works, we have analysed in detail sperm nucleomorphogenesis of *Octopus* and *Eledone*, and the primary structures of proteins responsible for chromatin condensation of spermiogenic cells. In these works, we have given evidence supporting that a limited number of changes in spermiogenic development can lead to important modifications in the nuclear shape of mature sperm. In fact, spermiogenesis is caused by a complex process of progressive activation of a genetic program (Sassone-Corsi, 2002; Kimmins et al., 2004), and therefore of dynamic interactions, ordered in time, between cellular elements. Thus, changes in the appearance of a specific element in this program, as well as changes in the moment of its activation, can produce critical modifications to the final result of spermiogenesis.

The present article shows a detailed analysis of sperm nucleomorphogenesis in *Sepia officinalis*, allowing a better understanding of the processes responsible for the evolutionary changes to the cephalopod sperm cell. The general spermiogeneses of *Sepia* and other cephalopods were first studied by Longo and Anderson (1970), Maxwell (1974, 1975), Fields and Thompson (1976), Arnold and Arnold (1978), Healy (1990a, 1990b, 1993), Selmi (1996) and Giménez-Bonafé et al. (2002a, 2002b).

2. Materials and methods

2.1. Animals

The various specimen of *S. officinalis* studied in this work were collected along the Catalonian coast of the Mediterranean Sea (Northeast of Spain). Gonads were dissected and treated immediately. The species *S. officinalis* (formerly *Eusepia officinalis*), belongs to the class Cephalopoda, order Decapoda, family Sepiidae.

2.2. Electron microscopy and confocal immunofluorescence

Conventional transmission electron microscopy was performed according to the method described in Giménez-Bonafé et al. (2002a). Briefly, portions of male gonads were fixed in 2.5% glutaraldehyde, 0.1 M cacodylate buffer, pH 7.4, and postfixed in osmium tetroxide in cacodylate buffer. Following fixation, the samples were dehydrated and embedded in Spurr's resin. Sections were stained with uranyl acetate and lead citrate and observed on a Hitachi 4-600 transmission electron microscope (Hitachi Ltd., Tokyo, Japan).

Immunomicroscopy was performed as in Martínez-Soler et al. (2007). Specifically, male gonad from *S. officinalis* was fixed in 4% paraformaldehyde with 1% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4, followed by a second fixation in which the glutaraldehyde was omitted. The sample was dehydrated and embedded in Lowicryl K4M resin. Ultrathin sections were applied to nickel grids, and were treated by flotation with anti-α or anti-β tubulin antibodies (Amersham Biosciences) prepared 1:500. An anti-mouse secondary antibody conjugated with 15 nm colloidal gold was used for indirect antibody detection, prepared 1:25. Prior to immunoelectron microscopy observation, the samples were contrasted in the same way as those embedded in Spurr's resin.

Optical immunofluorescence has been performed based on the technique of Zhao et al. (2004) with some variations, and has been used for the inset in Fig. 10. Semi-thin tissue sections (0.75 to 1 μm thick) were blocked with phosphate buffered saline (PBS) containing 1% bovine serum albumin (BSA)/10% normal goat serum/20 mM Glycine/0.1% Tween-20 for 30 min. The slides were then incubated with anti-α-tubulin antibody (1:25), diluted in the same solution used for blocking, with the glycine omitted, and incubated overnight at 4 °C. Samples were washed several times with PBS/0.1% Tween-20, followed by PBS. They were then incubated for 1.5 h with the secondary antibody, an Alexa Fluor 488 conjugated goat anti-rabbit (Molecular probes) prepared 1:500 in PBS/0.1% Tween-20/1% BSA/1% normal goat serum. Then the nuclei were stained with the far-red fluorochrome TO-PRO-3 (Molecular Probes) prepared 1:6000 in PBS, and washed in PBS before mounting with Immunofluore mounting media (MP Biomedicals).

3. Results

3.1. A. Correlation between the pattern of chromatin condensation and nuclear shape during spermiogenesis of S. officinalis (Fig. 1).

With the objective of determining if nucleomorphogenesis of *S. officinalis* is conditioned by the type of chromatin condensation, we examined the correlation between changes in the chromatin structure and changes in the spermiogenic nuclear shape.

The first change in chromatin structure is produced when somatic-like chromatin of the earliest spermatid nucleus is transformed into homogeneously packaged granules (actually, a fibrogranular structure) approximately 20 nm in diameter (Fig. 1A and B). This first structural remodelling is not correlated with important changes in nuclear shape or volume. In Fig. 1A and B, it is observed that the spermatid nucleus maintains an approximately spherical shape, and that aside from the apparent differences determined by the