ACTIN IN SPERMATIDS AND SPERMATOZOA OF *TELADORSAGIA CIRCUMCINCTA* AND *TRICHOSTRONGYLUS COLUBRIFORMIS* (NEMATODA, TRICHOSTRONGYLIDA)

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Summary :

Trichostrongyle nematodes provide valuable models for the study of nematode sperm, because their male germ cells are large and elongate, allowing easy identification of cell organelles. A previous study led to the unexpected result that actin co-localizes with MSP (Major Sperm Protein) in spermatids and spermatozoa of *Heligmosomoides polygyrus*. Actin expression in male germ cells of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* was studied using a monoclonal anti-actin antibody. Actin was demonstrated in the « fibrous bodies » in spermatids and in an anterior cap in spermatozoa. The actin labelling pattern in the two species studied was similar to that found in *H. polygyrus*, suggesting that this distribution of actin might be general for nematode male germ cells.

KEY WORDS : spermatozoon, actin, Nematoda, *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*, *Heligmosomoides polygyrus*

Résumé : L'ACTINE DANS LES SPERMATIDES ET LES SPERMATOZOÏDES DE *TELADORSAGIA CIRCUMCINCTA* ET *TRICHOSTRONGYLUS COLUBRIFORMIS* (NEMATODA, TRICHOSTRONGYLIDA)

Les nématodes Trichostrongylida sont des modèles intéressants pour l'étude des spermatozoïdes de nématodes, parce que leurs cellules germinales mâles sont allongées et de grande taille, ce qui permet une identification facile des organites. Une étude antérieure a montré de manière inattendue que l'actine a la même localisation que la MSP (Protéine Spermatique Majeure) dans les spermatides et les spermatozoïdes de Heligmosomoides polygyrus. *L'expression de l'actine a été étudiée dans les cellules germinales mâles de* Teladorsagia circumcincta *et* Trichostrongylus colubriformis *en utilisant un anticorps monoclonal anti-actine. La présence d'actine a été démontrée dans les « corps fibreux » des spermatides et dans le capuchon antérieur des spermatozoïdes. Le marquage anti-actine dans les deux espèces étudiées était similaire à celui décrit chez* H. polygyrus, *ce qui suggère que cette distribution de l'actine pourrait être générale dans les cellules germinales mâles de nématodes.*

MOTS CLÉS : *spermatozoïde, actine, Nematoda,* Teladorsagia circumcincta, Trichostrongylus colubriformis, Heligmosomoides polygyrus

INTRODUCTION

Spermatozoa of nematodes are devoid of a flagellum and show amoeboid movements (references in Mansir & Justine, 1995). The protein associated with this movement is not actin but the nematode sperm specific major-sperm-protein or MSP (Theriot, 1996). In *Caenorhabditis elegans* (Nelson, Roberts & Ward, 1982) and *Ascaris* (Nelson & Ward, 1981), the amount of actin is very low in spermatozoa. A recent study (Mansir & Justine, 1996) has shown that *Heligmosomoides polygyrus*, a trichostrongyle nematode, has large spermatids and spermatozoa in which cell organelles can be easily identified. In this species, immunocytochemistry observations were performed with anti-actin and anti-MSP antibodies, previously shown by immunoblotting to be strictly specific for their respective targets. A clear, but unexpected, result of immunocytochemistry was that actin co-localizes with MSP in spermatids and spermatozoa. Moreover, the same localization of actin in spermatids was also detected by fluorescent phalloidin, which is specific to F-actin, and the actin-associated protein tropomyosin was detected in spermatozoa in the same region as actin. Comparative studies have therefore been undertaken in two other species of trichostrongyle nematodes (*Trichostrongylus colubriformis* and *Teladorsagia circumcincta*) with similar sperm morphology. In this paper, we confirm the results on actin localisation previously obtained in the male germ cells of *Heligmosomoides polygyrus* by similar results obtained in these two species.

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MATERIAL AND METHODS

Specimens of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* were respectively obtained from experimentally infested rabbits and sheep. Germ cells were obtained by dissecting each male in a drop of PBS (phosphate buffer saline, Sigma) on a pit slide. The genital system was gently pressed to release germ cells, which adhered to the slide without coating. The pits were dried for one hour. Previous experiments with *Heligmosomoides polygyrus* have shown that the air-drying method of fixation caused artefacts at the level of the nucleus (Mansir & Justine, 1996), but that determination of the localization of protein by immunocytochemistry was not affected. Cells were processed at room temperature, fixed in acetone for 10 min, and rinsed in PBS (3 × 5 min). Non-specific antigens were blocked with 2 % Bovine Serum Albumin (Sigma) in PBS (BSA-PBS) for 45-90 min. A monoclonal anti-actin antibody developed against chicken gizzard actin (Amersham N 350) diluted at 1/100 or 1/400 in BSA-PBS or PBS was applied for 40 min. After washing (PBS 3 × 5 min) the goat anti-mouse FITC-conjugated antibody (Nordic, 1/40 in PBS) was applied for 40 min and then washed (PBS 3 × 5 min). Counterstaining was performed with Evans Blue (2 ‰ in PBS, 10 min). After a final wash (PBS 3 × 5 min), the preparation was mounted in Citifluor (Citifluor Ltd, London, UK). Controls were done by omitting the first antibody; they were negative and thus are not further illustrated or mentioned. Observations were made with an Olympus BH-2 epifluorescence microscope. Scanning electron microscope observations of sperm were performed with a routine method of glutaraldehyde fixation followed by dehydration in ethanol (Mansir & Justine, 1996).

OBSERVATIONS

The morphology of trichostrongyle germ cells is characteristic (Fig. 1). They are elongate cells (15-20 µm in length, 3-5 µm in width). The anterior extremity, or cap, is slightly wider than the rest of the cell and has a smooth surface. The rest of the cell shows an irregular surface with folds and pits. The pyriform nucleus, about 3 µm in length and 1.5 µm in width in its anterior wide part, is located at the posterior extremity of the cell. Anti-actin immunolabelling of *Te. circumcincta* male germ cells (Figs. 2A-D) showed a differential actin distribution depending upon the differentiation stage. Round cells (spermatocytes) attached to a rachis showed a general cytoplasmic labelling (Figs. 2A, B). Spermatids, either attached to a central rachis (Fig. 2C) or free (Fig. 2D) showed an intense labelling of the cytoplasm, consisting of numerous large dots. Late spermatids were less strongly labelled (Fig. 2D). Germ cells of *Tr. colubriformis* also showed different labelling patterns during spermiogenesis (Figs. 2E, F). A dot-like pattern of actin distribution was found in the cytoplasm (Fig. 2E), but spermatozoa showed an intense labelling of the cap opposite the nucleus and a weaker labelling of the cytoplasm (Fig. 2E). In this species, it was possible to label the spermatozoa packed in the genital tract, in regions were the sheath of the tract was disrupted (Fig. 2F).

DISCUSSION

In spermatids of *H. polygyrus*, a previous study (Mansir & Justine, 1996) has demonstrated, by the use of electron microscopy and immunocytochemistry, that the actin-containing structures in spermatids are the fibrous bodies. Fibrous bodies of nematode spermatids are a storage organelle for MSP, from which MSP is later released in spermatozoa to make the cytoskeleton of the pseudopods (Mansir & Justine, 1996; Noury-Sraïri, Gourbault & Justine, 1993; Ward & Klass, 1982). In spermatozoa of *H. polygyrus*, the actin labelling, and also the MSP labelling, is restricted to an anterior « cap », which is devoid of organelles (Mansir & Justine, 1996). The present study shows that a similar pattern is observed in *Tr. colubriformis* and *Te. circumcincta.*

The presence of actin in nematode spermatozoa is of functional interest. Studies on *C. elegans* and *Ascaris* have previously led to the conclusion that the amoeboid movement of sperm cells is mediated by MSP (Italiano, Roberts, Stewart & Fontana, 1996; Theriot, 1996) owing to the quasi-absence of actin in mature spermatozoa (Nelson *et al.*, 1982; Nelson & Ward, 1981). However, actin has been demonstrated in spermatids in several species of nematodes (Mansir & Justine, 1996; Nelson *et al.*, 1982; Noury-Sraïri *et al.*, 1993). The present study demonstrates actin in spermatids of two further species, thus indicating that previous results (Mansir & Justine, 1996) on *H. polygyrus* may be general for nematode sperm cells.

Trichostrongyles are useful models for the study of the cytoskeleton during nematode spermiogenesis because they have large spermatozoa with antero-posterior polarisation, which allow identification of organelles better than in *C. elegans* (Noury-Sraïri *et al.*, 1993). However, modification of spermatozoal morphology occurs in the female genital tract of trichostrongyle nematodes (Wright & Sommerville, 1984) and the experiments presented here should be extended to fully differentiated male germ cells. The pattern of actin

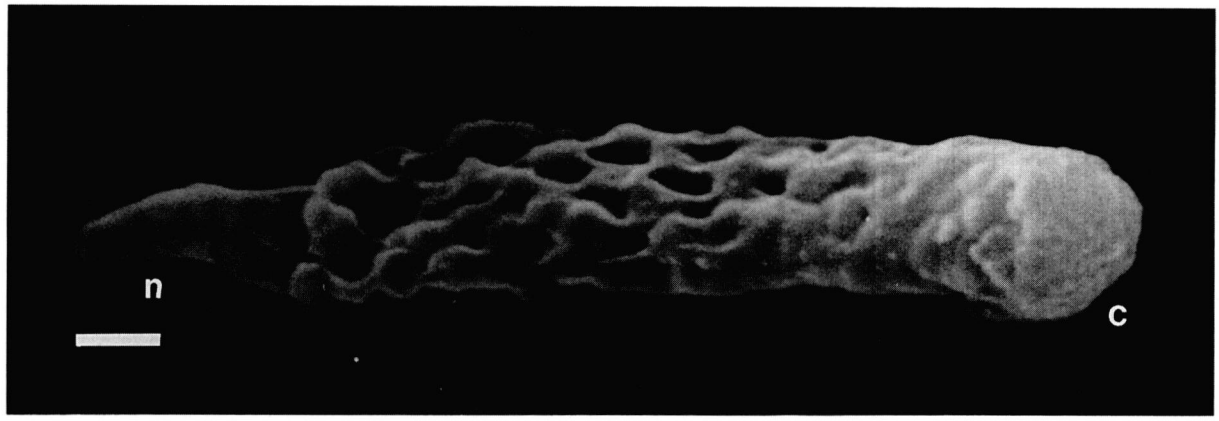

Fig. 1. — Scanning electron microscopy of a trichostrongyle spermatozoon, *Heligmosomoides polygyrus*. C, anterior cap; N, posterior nucleus. Scale bar 1 µm.

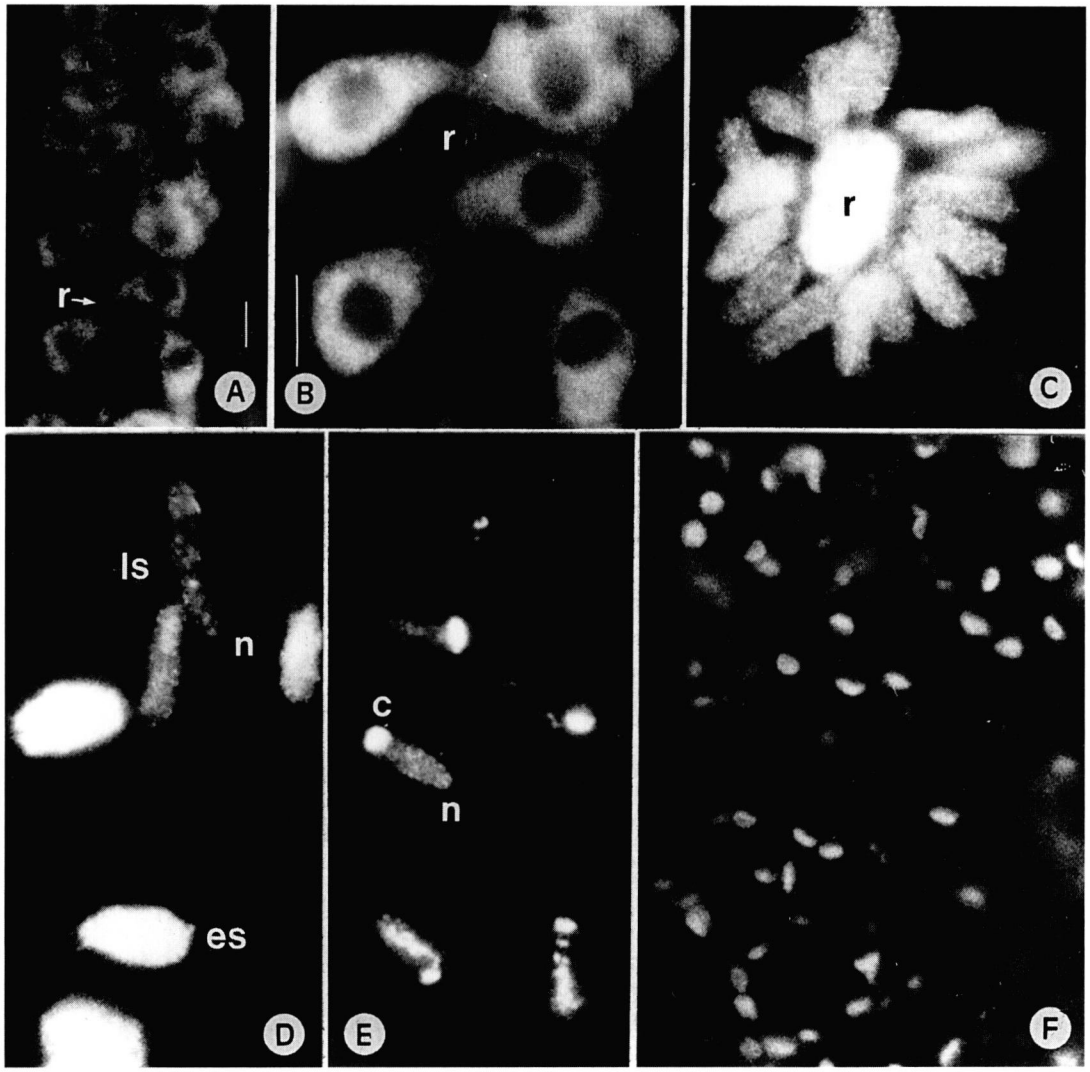

Fig. 2. — Actin labelling of germinal cells in *Teladorsagia circumcincta* (A-D) and *Trichostrongylus colubriformis* (E, F). A, B, round cells (probably spermatocytes) attached to rachis (r). C, early spermatids attached to rachis (r). The rachis (r) is counterstained by Evans blue, but this labelling does not correspond to the presence of actin in the rachis. Spermatids are labelled by the anti-actin antibody as dots, corresponding to the fibrous bodies. D, late spermatids and spermatozoa. Early spermatids (es) show a strong homogeneous labelling, late spermatids (ls) are slightly labelled. n, location of nucleus (not labelled). E, late spermatids heavily labelled at the level of the anterior cap (c) opposite the nucleus (n). F, spermatozoa packed in the genital tract, at a level with its sheath disrupted. Only the caps of spermatozoa are labelled. Scale bars 10 µm, for B-F bar in B.

labelling in the three species studied is similar, thus indicating common characteristics of the cytoskeleton, but shows some species-specific differences, possibly of taxonomic interest.

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