MFP1/MSD-1 and MFP2/NSPH-2 co-localize with MSP during C. elegans spermatogenesis

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Abstract

Until recently, the only verified component of Fibrous Bodies (FBs) within Caenorhabditis elegans spermatogenesis was the Major Sperm Protein (MSP), a nematode-specific cytосkeletal element. Earlier studies in the pig parasite Ascaris suum had identified accessory proteins that facilitate MSP polymerization and depolymerization within the pseudopod of crawling spermatozoa. In this study, we show that C. elegans homologs of the two Ascaris accessory proteins MFP1 and MFP2 co-localize with MSP in both the pseudopods of C. elegans sperm and the FBs of C. elegans spermatocytes.

Figure 1: (A) Overview of spermatogenesis and fibrous body–membranous organelle (FB-MO) development. (Top) Spermatocytes develop on a syncytial rachis then detach to undergo the meiotic divisions (1-3). Within meiotic prophase I, chromatin condenses into the 2300 chromosome bivalents. During the meiotic divisions (4-5), the bivalents separate into the 2300 chromosomes which are then condensed into the 2300 spermatids. (Bottom) Within meiotic prophase I, chromatin condenses into the 2300 chromosome bivalents. During the meiotic divisions (4-5), the bivalents separate into the 2300 chromosomes which are then condensed into the 2300 spermatids. (B) Alignment of MSP sequences from C. elegans ZK265.3/NSPH-2 (Nematode Specific Peptide family, group H) and C04G2.9/NSPH-3.2 with Ascaris suum MFP1/2 (MSP Fiber Protein) performed using the EMBL-EBI Clustal Omega Multiple Sequence Alignment tool (Madeira et al., 2019). Peptide for the anti-NSPH-2 antibody is underlined. (C-D) Anti-NSPH-2 labelling in hermaphrodites with genetic knockouts of nsph-2 (n=63, 9 experiments), (F) anti-NSPH-2 labelling (n=50, 7 experiments), and (G) labelling against the MFP1 homolog, MSD-1 (Major Sperm protein Domain containing) (n=46, 3 experiments). (H) Co-labeling of MSP with either (H) anti-NSPH-2 or (I) anti-MSD-1. In spermatogenesis (enlarged), the patterns were either fully (top) or partially overlapping (bottom). For NSPH-2-MSP n=16; for MSD-1/MSP n=14. Scale bars = 10 mm for C, D and E-G left; 5 mm for H, I and E-G right. Abbreviations: Karyosome spermatocytes (K), Metaphase spermatocytes (M), Budding Figures (B), Spermatids (S), and Spermatozoa (Z).

Description

During the process of cell differentiation, specific cytoskeletal proteins can sequentially assemble into a wide variety of diverse molecular superstructures. Nematode spermatogenesis provides a powerful system for studying these transitions since sperm-specific transcription ceases prior to the meiotic divisions and translation ceases shortly thereafter (Chu and Shakes, 2013). Therefore, structural transitions that follow the meiotic divisions must be carried out by the remodeling of already synthesized proteins. The Major Sperm Protein (MSP) is a nematode-specific cytoskeletal element whose polymerization dynamics drive the pseudopodal-based motility of the activated sperm (Roberts, 2005). In C. elegans, MSP additionally functions as the extracellular signaling molecule for triggering both ovulation and oocyte maturation (Miller et al., 2019). Proteins for the anti-NSPH-2 antibody is underlined. (C-D) Anti-NSPH-2 labelling in hermaphrodites with genetic knockouts of nsph-2 (n=63, 9 experiments), (F) anti-NSPH-2 labelling (n=50, 7 experiments), and (G) labelling against the MFP1 homolog, MSD-1 (Major Sperm protein Domain containing) (n=46, 3 experiments). (H) Co-labeling of MSP with either (H) anti-NSPH-2 or (I) anti-MSD-1. In spermatogenesis (enlarged), the patterns were either fully (top) or partially overlapping (bottom). For NSPH-2-MSP n=16; for MSD-1/MSP n=14. Scale bars = 10 mm for C, D and E-G left; 5 mm for H, I and E-G right. Abbreviations: Karyosome spermatocytes (K), Metaphase spermatocytes (M), Budding Figures (B), Spermatids (S), and Spermatozoa (Z).
[Reagents Table]

**Strain** | **Genotype** | **Available from**
--- | --- | ---
N2 | Wild-type C. elegans strain | CGC
CB488 | him-5(e1490) V | CGC
JDW/307 | nph-3.2(wdrf6)[C04G2.9::exon 1 STOP] | J. Ward lab
JDW/308 | nph-2(wdrf7)[ZK265.3::exon 1 STOP] | J. Ward lab
**Plasmid** | **Genotype** | **Description**
pRF4 | co-injectable marker – dominant roller | 4 kb fragment of genomic DNA from rol-6(u1006) collagen gene in Bluescript vector (Mello et al., 1991).

**Antibody**

- **anti-MSP**: Mouse monoclonal, 4D5 (Kosinski et al., 2005)
- **anti-MSD-1**: Rabbit polyclonal, R194P (Kosinski et al., 2005)
- **anti-NSPH-2**: Rabbit polyclonal (Dokshin et al., 2018; Wang et al., 2018). A BamHI site was included 3' to the stop codons to facilitate genotyping. Strains JDW/307 nph-3.2[wdrf6][C04G2.9::exon 1 STOP] and JDW/308 nph-2[wdrf7][ZK265.3::exon 1 STOP] were generated by CRISPR injection of an RNP complex [250 ng/µl of a blasticum Cas9 protein (Zarais et al., 2015)] into the relevant RNP [250 ng/µl of the relevant crRNA, and 100 ng/µl tracrRNA that was first incubated at 37°C for 15 minutes, then mixed with the coinjection marker pRF4 (50 ng/µl) and the relevant repair oligonucleotide (110 ng/µl)].

**Antibodies and immunocytochemistry**

Sperm spreads were obtained by dissecting 8-15 male worms per slide in 7.5 microliters of egg buffer (Edgar, 1995) onto ColorFrost Plus slides (Fisher Scientific, 12-550) coated with poly-L-lysine (Sigma Aldrich, P8290). Light pressure was applied to coverslips to flatten the samples. Samples were then freeze cracked in liquid nitrogen and fixed overnight at −20°C methanol.

**Images were acquired with epifluorescence using an Olympus BX60 microscope equipped with a QImaging EXi Aqua CCD camera. Photos were taken, merged, and exported for analysis.**

**Methods**

**Request a detailed protocol**

**Biorh culture**

Worms were cultured on MYOB plates (Church et al., 1995) and inoculated with the C. elegans strain OP50, using methods similar to those described by Brenner (1974).

**Creation of knock-out lines**

CRISPR/Cas9 mutation was performed as previously described (Paix et al., 2014; Dokshin et al., 2018; Wang et al., 2018). Briefly, C. elegans strain N2 was gene-edited by the insertion of a 43-base-pair sequence, which includes multiple stop codons in all three reading frames to disrupt translation (Wang et al., 2018). A BamHI site was included 3’ to the stop codons to facilitate genotyping. Strains JDW/307 nph-3.2[wdrf6][C04G2.9::exon 1 STOP] and JDW/308 nph-2[wdrf7][ZK265.3::exon 1 STOP] were generated by CRISPR injection of an RNP complex (250 ng/µl of a blasticum Cas9 protein (Zarais et al., 2015)] into the relevant RNP [250 ng/µl of the relevant crRNA, and 100 ng/µl tracrRNA that was first incubated at 37°C for 15 minutes, then mixed with the coinjection marker pRF4 (50 ng/µl) and the relevant repair oligonucleotide (110 ng/µl)] before being injected into wild-type L4 + 1 day worms grown at 20°C. After 4 days, rolling worms were plated individually, allowed to lay eggs, and the parental animal was genotyped by PCR and BamHI digestion. For candidate knock-ins the extrachromosomal array was removed by selecting non-resistant L4 + 1 day worms grown at 20°C. After 4 days, rolling worms were plated individually, allowed to lay eggs, and the parental animal was genotyped by PCR and BamHI digestion. For candidate knock-ins the extrachromosomal array was removed by selecting non-resistant L4 + 1 day worms grown at 20°C.

**Sperm spreads**

Sperm spreads were obtained by dissecting 8-15 male worms per slide in 7.5 microliters of egg buffer (Edgar, 1995) on ColorFrost Plus slides (Fisher Scientific, 12-550) coated with poly-L-lysine (Sigma Aldrich, P8290). Light pressure was applied to coverslips to flatten the samples. Samples were then freeze cracked in liquid nitrogen and fixed overnight at −20°C methanol.

**Imaging and analysis**

Images were acquired with epifluorescence using an Olympus BX60 microscope equipped with a QImaging EXi Aqua CCD camera. Photos were taken, merged, and exported for analysis using the program ImageJ. For control studies (Fig. 1C-D), image exposures were kept constant with no further image processing. For other images, image exposures were optimized for individual gonads. The levels adjust function in Adobe Photoshop was used to spread the data containing regions of the image across the full range of tonalities.
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References


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