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 Caenornabditis elegans spermatocytes was the Major Sperm Protein (MSP), a nematode-specific cytoskeletal 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. (Bottom) Within meiotic phases II, components which are no longer needed partition to a central residual body (RB). (B) Spermatocytes develop on a syncytial rachis then detach to undergo the meiotic divisions. (Bottom) Within meiotic phases II, components which are no longer needed partition to a central residual body (RB). (C) Left to right developmental sequence of developing spermatocytes and spermatids. (D) Hermaphrodite C. elegans spermatogenesis and fibrous body–membranous organelle (FB-MO) complexes (Fig. 1A, Roberts et al., 2005). (E-G) Sperm spreads and individually staged cells with (E) anti-MSP labelling (n=50, 7 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) H2O2-sulphating of sperm with either (H) anti-NSPH-2 or (I) anti-MSD-1. In spermatozoa (enlarged), the patterns were either fully (top) or partially overlapping (bottom). For NSPH-2/MSD-1 labelling (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). (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. (Bottom) Within meiotic phases II, components which are no longer needed partition to a central residual body (RB). (Bottom) Within meiotic phases II, components which are no longer needed partition to a central residual body (RB).

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 pseudopod-based motility of the activated sperm (Roberts, 2005). In C. elegans, MSP additionally functions as the extracellular signalling molecule for triggering both ovulation and oocyte maturation (Miller et al., 2003). MSP is highly abundant in sperm, where it reaches 10-15% of total and 40% of soluble cellular protein (Roberts et al., 2005). Within developing spermatocytes, MSP is packaged into fibrous body–membranous organelle (FB-MO) complexes (Fig. 1A, Roberts et al., 1986). By assembling into a paracrystalline array, FBs ensure the critical meiotic processes of chromosome segregation and cytokinesis while also being packaged for efficient segregation into spermatids during the post-miotic partitioning process (Chu and Shakes, 2013, Nishimura and L’Hernault, 2010, Price et al., 2021). Following the meiotic divisions and sperm individualization, FBs disassemble, and MSP disperses as dimers throughout the spermatid cytoplasm (Fig. 1A). When sperm activate to form motile spermatozoa, MSP polymerization within the pseudopod drives the motility of the activated sperm (Chu and Shakes, 2013). Thus, MSP exists in at least three distinct molecular states: 1) in highly organized paracrystalline FBs within developing spermatocytes 2) as unpolymerized dimers within spermatids, and 3) in dynamically polymerizing filaments and fibers within crawling spermatozoa. Because MSP neither binds nucleotides nor assembles into polar filaments, its assembly and disassembly dynamics require accessory proteins. Previous biochemical studies in the pig parasite Ascaris suum identified two 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.
Genotype
Rabbit polyclonal
CGC
J. Ward lab
Goat anti-rabbit IgG
nsph-2 (wrd57[ZK265.3::exon 1]

Rabbit antiserum was generated by immunizing a rabbit against the aa 92-108 (EWLPLKQRHENNEGIRE) of Jackson ImmunoResearch, 115-545-146

Wildtype
him-5 (e1490)
Rabbit polyclonal, R194P
Invitrogen, A32732

Available from
7/22/2021 -

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 iVision. For control studies (Fig. 1C-D), image exposures were kept constant with no further image processing. For others, image exposures were optimized for individual samples.

Imaging and analysis

Components of the FBs include MFP1/NSPH-2 and MSD-1, which are important for spermatid motility. Our discovery that MSD-1 and MFP2/NSPH-2 are non-essential may be due to the fact that MSD-1 and NSPH-2 are packaged together with MSP in the FBs of developing spermatocytes supports a model in which the FBs function to gather, concentrate, and sequester proteins that will ultimately drive or regulate pseudopod motility. While early studies of MSP dynamics in Ascaris capitalized on its assets for biochemical approaches, parallel genetic approaches in C. elegans have studied the function of MSP family members in spermatogenesis. Here we show that two of these motor proteins, MSD-1 and NSPH-2, are packaged together with MSP in the FBs of C. elegans spermatocytes. Our discovery that MSD-1 and MFP2/NSPH-2 are packaged together with MSP in the FBs of developing spermatocytes supports a model in which the FBs function to gather, concentrate, and sequester proteins that will ultimately drive or regulate pseudopod motility.

Goat anti-mouse IgG
CGC

Plasmid

Antibodies and immunocytochemistry

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, P6290). Light pressure was applied to covergrips to flatten the samples. Samples were then freeze cracked in liquid nitrogen and fixed overnight in –20°C methanol. Specimen preparation and antibody labeling followed established protocols (Shakes et al., 2009). Primary antibodies included: 1:150 4DS mouse anti-MSP monoclonal (Kosinski et al., 2005) and 1:200 rabbit anti-NSPH-2 rabbit polyclonal (Kosinski et al., 2005). Affinity purified rabbit antiserum against MFP2/NSPH-2 (ZK265.3) was generated by YenZym Antibodies using the peptide 92-108 EWLPLKQRHENNEGIRE. In experiments, the antibody was used at a 1:200 dilution. All samples were incubated with primary antibodies for 2 hours at room temperature. Affinity-purified secondary antibodies included 1:400 Alexa Fluor Plus 555 goat anti-rabbit IgG (Invitrogen, A32752) and 1:300 Alexa Fluor 488 goat anti-mouse IgG (H+L) (Jackson Immunoresearch, 115-545-146). Final slides were mounted with Fluoro Gel with DABCO (Electron Microscopy Sciences #17985-02) containing DAPI.

Antibodies

Our discovery that MSD-1 and MFP2/NSPH-2 are non-essential may be due to the fact that MSD-1 and NSPH-2 are packaged together with MSP in the FBs of developing spermatocytes supports a model in which the FBs function to gather, concentrate, and sequester proteins that will ultimately drive or regulate pseudopod motility.

CRISPR technologies give us the ability to knockout multiple genes and explore MSP dynamics in new ways. Beyond MSD and NSPH, will other proteins first identified in Ascaris as regulators of MSP dynamics and sperm motility (Buttery et al., 2003) also localize to the FBs of developing spermatocytes? Alternatively, are there three more proteins like the intrinsically disordered protein SPE-18 that localize to FBs and facilitate their growth and shaping but then are degraded shortly after the meiotic divisions (Price et al., 2021)? Further studies will reveal whether MSD-1 and NSPH-2 function together with SPE-18 to facilitate FB assembly, or if they are just conveniently packaged in FBs alongside MSP.

Methods

Request a detailed protocol

Biorh culture

Worms were cultured on MY08 plates (Church et al., 1995) and inoculated with the E. coli strain OP50, using methods similar to those described by Brenner (1974).

Creation of knock-out lines

CRISPR/Cas9 mutagenesis was performed as previously described (Paix et al., 2014; Dokshit et al., 2018; Wang et al., 2018). Briefly, 264.5C. elegans strain N2 was gene-edited by the insertion of a 43-base pair sequence that includes multiple stop codons in all three reading frames to disrupt translation (Wang et al., 2018). A BamiHI site was included 3’ to the stop codons to facilitate genotyping. Strains JDW307 (nspf-2[wrd65(C04C2.9::exon 1 STOP)]) and JDW308 (nspf-2[wrd77(ZK265.3::exon 1 STOP)]) were generated by CRISPR injection of an RNP complex (250 ng/μl of guide and 150 ng/μl of Cas9 protein) (Zaruba et al., 2015). 50 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 master prF4 (50 ng/μl) and the relevant repair oligo (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 BamiHI digestion. For candidate knock-ins the extrachromosomal array was removed by selecting non-roller progeny. Genotyping primer sequences can be provided upon request. The tracrRNA and crRNAs were obtained from Integrated DNA Technologies (IDT).

Repair oligos (BamHI sites in bold)

C04229.2 crRNA: 5’-TACGGCGTTCGCGAGGAAGG-3’
ZK265.3 crRNA: 5’-AAGGAAAACTGTAGTTGGG-3’

Repair oligos (BamHI sites in bold)

C04229.2 crRNA: 5’-TACGGCGTTCGCGAGGAAGG-3’
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Reagents

Strain
N2
CB4088
JDW307
JDW308
phsp-3.2
phsp-3.2
plasmid
pRF4
Antibody
anti-MSP
anti-NSPH-2
Alexa Fluor Plus 555
 Alexa Fluor 488

Genotype
Wildtype C. elegans strain
him-5(e1490) V
nspf-2(wrd65[C04C2.9::exon 1 STOP])
nspf-2(wrd77[ZK265.3::exon 1 STOP])

Description
4 kb fragment of genomic DNA from rol-6(eu1006) collagen gene in Bluescript vector (Mello et al., 1991).

Antibody

Animal and clonality
Mouse monoclonal, 4DS
Rabbit polyclonal, R194P
Rabbit polyclonal
Goat anti-rabbit IgG
Goat anti-mouse IgG

Available from
CGC
CGC
J. Ward lab
J. Ward lab

Author contributions

YH, CM, and TM conceived and designed the experiments. YH performed the experiments and analyzed the data. YH, CM, and TM wrote the paper.

Acknowledgments

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References


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Author Contributions: Kayleigh N. Morrison: Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. Christopher M. Uyehara: Formal analysis, Investigation, Methodology, Validation, James Matthew Ragle: Investigation, Methodology, Writing - review and editing. Jordan D. Ward: Funding acquisition, Supervision, Writing - review and editing. Diane C. Shakes: Conceptualization, Funding acquisition, Supervision, Writing - review and editing.

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