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 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. Within meiotic prophase II, components which are no longer required for FBs develop on the cytosolic face of the Golgi-derived membranous organelle (MO). During the budding division, FB-MO complexes partition to the spermatids. MOs then dock with the plasma membrane as the FBs disassemble and release MSP (Major Sperm Protein) dimers into the cytosol. During sperm activation, MSP localizes to the pseudopod, and MOs fuse with the plasma membrane. (B) Alignment of Ascaris MFP2 with the C. elegans homologs ZK265.3/NSPH-2 (Nematode Specific Peptide family, group H) and C04G2.9/NSPH-3.2 with Ascaris MFP2 (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) DAPI and anti-NSPH-2 labelling in spermatocytes. (E) Sperm spreads and individually staged cells with (E) anti-MSP labelling in C. elegans; (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-J) Co-labelling of MSP 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/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), Developing spermatids (D), Mature spermatids (Z), Nuclear remnants (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 signaling 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, 2005). Within developing spermatocytes, MSP is packaged into fibrous body–membranous organelle (FB-MO) complexes (Fig. 1A, Roberts et al., 1986). By assembling into paracrystalline FBs, MSP is both sequestered away from the critical meiotic processes of chromosome segregation and cytokinesis while also being packaged for efficient segregation into spermatids during the post-meiotic partitioning process (Chu and Shakes, 2013, Nishimura and L’Hermant, 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). At sperm activation to form motile spermatozoa, MSP polymerization within the pseudopod drives the motile pseudopodal dynamics (Chu and Shakes, 2015). 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 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 spermatozoa.
similarity with each other. Ascaris MFPI has two isoforms, MFPI-a and MFPI-b, which when aligned with C. elegans MSD-1 (Major Sperm protein Domain containing), show 52% identity and 70.8% sequence similarity, and 51.9% identity and 66.0% sequence similarity, respectively (Buttery et al., 2003). The sequence similarity between A. suum and C. elegans motility proteins suggests that they may have similar functions and binding properties.

To determine where NSPH-2 (MFPI) and MSD-1 (MFPI) localize within C. elegans spermatoocytes and spermatan, we performed immunocytochemistry experiments. For our analysis, we generated a rabbit polyclonal antibody against a unique NSPH-2 peptide (Fig. 1B) and obtained a polyclonal antibody against C. elegans MSD-1, from David Greenstein (Kosinski et al., 2005). To test the specificity of the new anti-NSPH-2 antibody, we used high-efficiency genome editing to generate CRISPR-Cas9 knockout (KO) lines through insertion of multiple premature termination codons in all three frames for both ZK265.3 (npk-2) and C04G2.9 (npk-2) (Wang et al., 2018). Although neither knockout strain on its own exhibited a phenotype, they were useful for determining the specificity of our anti-NSPH-2 antibody. In control npk-2 KO hermaphrodites, the anti-NSPH-2 antibody labeled distinct structures within late-stage spermatoocytes (Fig. 1C) as well as the pseudopods of spermatoocytes within the spermatheca (Fig. 1D). In npk-2 KO hermaphrodites, only background labeling was observed. We then compared the localization of anti-NSPH-2 and anti-MSD-1 with the well-characterized anti-MSP pattern in male gonads (Fig. 1E-G). In spermatoocytes, both NSPH-2 and MSD-1 antibodies localized to the pseudopod, and matched the known pattern in Ascaris spermatoocytes. Within prophase and meiotically dividing spermatoocytes, NSPH-2 and MSD-1 antibodies labeled discrete structures throughout the cytoplasm in a pattern strongly resembling FBs labeled by MSP antibodies (Fig. 1E-G). To verify that NSPH-2 and MSD-1 localized to FBs, we co-labeled male germ lines with anti-MSF and either anti-NSPH-2 or anti-MSD-1 antibodies (Figure 1H-I). Within spermatoocytes, the fully overlapping patterns revealed that NSPH-2 and MSD-1 are backgound along with the FBs in the FBs. In spermatids, the MSP and MSD-1 were overlapping (Figure 1I). In contrast, except in immature spermatids that still retained their FBs, the NSPH-2 and MSP patterns only partially overlapped. In spermatids, the NSPH-2 labeling was notably less robust which may suggest that a change in protein conformation or binding partners is blocking the antigenic site. NSPH-2 does not appear to be degraded as it is present in the pseudopods of spermatozoa. All three antibodies labelled the pseudopods of spermatozoa, with the NSPH-2 and MSD-1 patterns either fully overlapping the MSP pattern (top set) or being restricted to a more central portion, adjacent to the cell body (bottom set).

Until recently, it was thought that Major Sperm Protein (MSP) was the only component of Flagellar Bodies (FBs) in C. elegans. Our discovery that MSD-1 and MFPI2/NSPH-2 are packages together with MSP in the FBs of developing spermatoocytes 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 were stymied by the fact that many key factors, including MSP, MFPI (MSD), and MFPI2 (NSPH) are encoded by multigene families. Our own individual knockout strains of npk-2 and npk-3J showed no obvious phenotype. This result does not mean that MFPI2/NSPH is non-essential, but that exploring its function may require knocking out combinations of up to five non-identical, sperm-related NSPH genes. Similarly, the four MSD genes encode identical proteins that may need to be deleted to generate a mutant phenotype. 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 spermatoocytes? Alternatively, are there 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., 2012)? 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

Biorhythm

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

Creation of knock-out lines

CRISPR/Cas9 mutagenesis 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 that includes multiple stop codons in all three reading frames to disrupt translation (Wang et al., 2018). A BanHI site was included 3’ to the stop codons to facilitate genotyping. Strands JDW307 npk-2/3 (from JDW306 [C04G2.9: exon 1 STOP]) and JDW308 npk-2/3 (from JK265.3: exon 1 STOP) were generated by CRISSPR injection of an RNP complex (250 ng/µl) of zinc-finger-Cas9 protein (Zarir 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 oligos (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 BanHI digestion. For candidate knock-in the extrachromosomal array was removed by selecting non-replicator progeny. Genotyping primer sequences can be provided upon request. The tracrRNA and crRNAs were obtained from Integrated DNA Technologies (IDT). C04G2.9 crRNA: 5’-TACCGGGCTCAGGGCAAGG-3’ ZK265.3 crRNA: 5’-AAGGAAAACGTGATTGGG-3’

Repair oligos (BanHI sites in bold)

C04G2.9::NSPH-3.2

AAAGACCTTGGGAGCTCAGGAAATCGGGGAAATGTTGCTCCGAGCGAGGTGACTAAGTGATAA ZK265.3::NSPH-2

TACAACAAAAATTGGAAGAAGAATCTGATTGGGAGCTCAGGAAATCGGGGAAATGTTGCTCCGAGCGAGGTGACTAAGTGATAA

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, P8290). Light pressure was applied to coverslips to flatten the samples. Samples were then freeze cracked in liquid nitrogen and fixed overnight in −20°C methanol. Sperm spreads were obtained by dissecting 8-15 male worms per slide in 7.5 microliters of egg buffer (Edgar, 1995) 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 in −20°C methanol.

Reagents

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Acknowledgments: We would like David Greenstein for supplying antibodies against MSP and MSD-1.

References


Funding: The work was supported by a grant R15GM-096309 from the National Institutes of Health and the McLeod Tyler Professorship to D.C.S., a National Science Foundation (NSF) Division of Molecular and Cellular Bionsciences CAREER award (1942922) to J.D.W., and an Honors Fellowship grant from the Charles Center at William & Mary to K.N.M.

Author Contributions: Kayleigh N. Morrison: Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. Christopher M. Uyehara: Formal analysis, Investigation, Methodology, Validation, James Matthew Raghe: 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.

Reviewed By: Anonymous

History: Received June 29, 2021 Revision received July 17, 2021 Accepted July 19, 2021 Published July 22, 2021

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Citation: Morrison, KN; Uyehara, CM; Ragale, JM; Ward, JD; Shakes, DC. (2021). MFP1/MSD-1 and MFP2/NSPH-2 co-localize with MSP during C. elegans spermatogenesis. microPublication Biology. https://doi.org/10.17912/micropub.biology.000427