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spe-43 is required for sperm activation in *C. elegans*

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ABSTRACT

Successful fertilization requires that sperm are activated prior to contacting an oocyte. In *C. elegans*, this activation process, called spermiogenesis, transforms round immobile spermatids into motile, fertilization-competent spermatozoa. We describe the phenotypic and genetic characterization of *spe-43*, a new component of the *spe-8* pathway, which is required for spermiogenesis in hermaphrodites; *spe-43* hermaphrodites are self-sterile, while *spe-43* males show wild-type fertility. When exposed to Pronase to activate sperm *in vitro*, *spe-43* spermatids form long rigid spikes radiating outward from the cell periphery instead of forming a motile pseudopod, indicating that spermiogenesis initiates but is not completed. Using a combination of recombinant and deletion mapping and whole genome sequencing, we identified F09E8.1 as *spe-43*. SPE-43 is predicted to exist in two isoforms; one isoform appears to be a single-pass transmembrane protein while the other is predicted to be a secreted protein. SPE-43 can bind to other known sperm proteins, including SPE-4 and SPE-29, which are known to impact spermiogenesis. In summary, we have identified a membrane protein that is present in *C. elegans* sperm and is required for sperm activation *via* the hermaphrodite activation signal.

1. Introduction

To fertilize an egg, most sperm must go through the processes of post-meiotic differentiation and activation whereby they gain polarity, motility, and fertilization competence. In *C. elegans* these processes occur concurrently and are referred to collectively as sperm activation or spermiogenesis. During spermiogenesis, round immotile spermatids transition to functional spermatozoa through the formation of a pseudopod, fusion of membranous organelles (MOs) with the plasma membrane, and sorting of proteins and organelles to either the pseudopod or the cell body (Ward et al., 1981, 1983). The initiation of spermiogenesis relies on extracellular signals, allowing for the timing to be precisely controlled in a sex-specific manner (Ellis, 2017). In addition, this signal is relayed through the spermatid without the synthesis of new RNAs or proteins (Ward, 1986). Thus, the study of *C. elegans* sperm activation examines many basic aspects of cell biology including cell signaling, changes in cellular physiology, and establishment of cell polarity through post-translational mechanisms.

Two separate pathways have been identified for the activation of *C. elegans* sperm *in vivo* (Ellis and Stanfield, 2014). Activation of hermaphrodite self-sperm is dependent on the "*spe-8* pathway". To date, this pathway includes five genes: *spe-8, spe-12, spe-19, spe-27,* and *spe-29*. The *spe-8* gene encodes a non-receptor tyrosine kinase, but the other four genes lack any identifiable functional domains. Mutations in any of these genes result in the same general phenotype: hermaphrodites do not produce self-progeny but males are fertile (Geldziler et al., 2005; Minniti et al., 1996; Muhlrad et al., 2014; Nance et al., 2000, 1999; Shakes and Ward, 1989). The extracellular trigger that signals through the *spe-8* group is not yet known. However, a serine protease, TRY-5, present in male seminal fluid was shown to activate sperm through a *spe-8*-independent pathway (Smith and Stanfield, 2011). Thus, male fertility is retained in the absence of any of the *spe-8* group components.

The current model is that SPE-8, SPE-12, SPE-19, SPE-27, and SPE-29 work together in a complex at the sperm plasma membrane where they receive and transduce an extracellular sperm activation signal (Ellis and Stanfield, 2014). This model is supported by data showing that SPE-8 is localized to the spermatid cell membrane and this localization is dependent on *spe-12, spe-19,* and *spe-27* (Muhlrad et al., 2014). Additionally, SPE-12, SPE-19, and SPE-29 all have transmembrane domains and a portion of SPE-12 has been shown to exist on the sperm cell surface (Nance et al., 1999).

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Here, we describe a new gene that is required for sperm activation. We find that *spe-43* displays a classic *spe-8* group phenotype where hermaphrodites are self-sterile due to defective spermiogenesis and males are fertile. Like *spe-12, spe-19,* and *spe-29*, the *spe-43* gene encodes a single-pass transmembrane protein and likely acts as a signaling component within the sperm to initiate sperm activation (Geldziler et al., 2005; Nance et al., 2000, 1999). The SPE-43 protein also contains a DX domain of currently unknown function. Binding studies show that SPE-43 can interact with the *spe-8* group member SPE-29, as well as other sperm proteins with roles in spermatogenesis, sperm activation, and sperm function. Our discovery of *spe-43* adds a new factor to an emerging pathway that cannot be fully understood without the full inventory of components.

2. Materials and methods

2.1. Strains and culture methods

General maintenance and crosses of *C. elegans* were performed as described previously (Brenner, 1974). Nematodes were reared at 20 °C unless otherwise indicated. Full descriptions of all genotypes used can be found at wormbase.org. Wild type refers to Bristol N2. The Hawaiian strain CB4856 was used for SNP mapping and creation of Hawaiian hybrids for whole-genome sequencing. The *spe-43(eb63)* strain was identified in an ethyl methanesulfonate (EMS) mutagenesis screen for genes with fertility defects by Dr. Andrew Singson while in the L'Hernault laboratory at Emory University. The *spe-43(jn5)* strain was identified by Amanda Mulia in the laboratory of Dr. Gillian Stanfield in an EMS screen for suppressors of *swm-1*. When we crossed *jn5* with *eb63*, the F_1 trans-heterozygous hermaphrodites remained self-sterile (Fig. 1A) indicating that they are both *spe-43* alleles.

2.2. Progeny/ovulation counts

To determine brood sizes and ovulation rates, L4 hermaphrodites were picked onto individual plates and allowed to self-fertilize. Hermaphrodites were transferred to fresh plates daily until new eggs and/or oocytes were no longer observed. Oocytes and/or progeny were counted daily. To assess male fertility, young adult males were crossed to L4 *dpy-5(e61)* hermaphrodites at 20 °C in a 4:1 ratio. After 48 h, the P_0 worms were removed from the plates. The number of outcross (non-Dpy) and self (Dpy) progeny were counted once the progeny were old enough to score for the Dpy phenotype. In a separate experiment, only the P_0 males were removed after 48 h and outcross progeny were counted daily until the *dpy-5* hermaphrodite stopped producing outcross progeny. Once the P_0 males were removed the *dpy-5* hermaphrodites were transferred onto new plates daily until progeny counts ceased. Hermaphrodites that died during the assay or went missing from the plate were excluded from the analysis. All errors reported are given as standard error of the mean.

2.3. Light microscopy

Differential interference contrast microscopy (DIC) images of live worms or dissected worms were obtained using a Zeiss Universal microscope and captured with an Optronics microscope camera using Magnafire image software (Karl Storz Industrial – America, Inc. El Segundo, CA) or a ProgRes camera (Jenoptik) using ProgResCapturePro software.

2.4. In vitro sperm activation

Males were picked at the L4 or young adult stage and isolated from hermaphrodites for 24 h. After 24 h the reproductive tract was dissected in pH 7.8 sperm medium (SM) both with and without the known *in vitro* activators Pronase (200 µg/ml) or triethanolamine (TEA, 120 mM at pH 7.8) (L'Hernault and Roberts, 1995; Ward et al., 1983). Hermaphrodites were picked at the L4 stage and isolated from males for 24 h after which the reproductive tract was dissected in pH 7.8 sperm medium with and without Pronase.

2.5. DAPI staining

To examine sperm cell nuclei, adult hermaphrodites were fixed in cold methanol for 30 s then placed in VECTASHIELD antifade mounting medium with DAPI (DAPI concentration is 1.5 µg/ml)(Vector Laboratories, Burlingame, CA) and mounted on 2% agarose slides for viewing using both fluorescence and Nomarski imaging.

2.6. Transactivation assay

spe-43 L4 hermaphrodites were crossed to *fer-1(hc1); him-5(e1490)* males in a 1:5 ratio at 24.5 °C, a temperature at which *fer-1(hc1)* worms are sterile due to a sperm motility defect (Shakes and Ward, 1989; Ward and Miwa, 1978). As controls, *fer-1(hc1); him-5(e1490)* males were crossed to *fer-1(hc1); him-5(e1490)* hermaphrodites. After 48 h the parents were removed from the plates. The number of progeny produced by each hermaphrodite during that 48 h period was counted after an additional 2 days.

2.7. spe-6 suppression

Epistasis experiments were performed using the weak hypomorphic allele *spe-6*(*hc163*) as described by (Geldziler et al., 2005; Muhlrad and Ward, 2002). Homozygous *spe-6(hc163) dpy-18(e364)* hermaphrodites were mated to either *spe-43(eb63)* males or *fog-2* males. *fog-2* was used as a control hermaphrodite-specific sterile mutation that cannot be suppressed by *spe-6(hc163)*. F_1 heterozygotes were allowed to self-fertilize. F_2 Dpy hermaphrodites were then picked and scored for sterility. To ensure that some of the F_2 Dpy hermaphrodites were homozygous for *spe-43*, individual F_2 Dpy hermaphrodites were then crossed to homozygous *spe-43* males and the F_3 progeny were scored for the *spe-43* phenotype (sterility). If both parents are homozygous for *spe-43*, then all of their progeny should also be homozygous for *spe-43*, and thus sterile. We observed that 2 of the 19 F_2 Dpy hermaphrodites crossed to *spe-43* males produced all sterile F_3 progeny, confirming that the *spe-43* homozygous class of animal was present amongst the F_2 animals.

2.8. Genetic mapping

Linkage, three-factor, and deficiency mapping were carried out as described in (Fay, D.). Linkage analysis placed *spe-43* on chromosome IV. Three factor mapping further localized *spe-43* to the right arm of chromosome IV. Briefly, *spe-43(eb63)* males were crossed to the strain MT4150 (*unc-17 dpy-4*), recombinants from among F_1 progeny were isolated, and the percent of recombinants was determined. Analysis of the recombinants placed *spe-43* between *unc-17* and *dpy-4*. Using deficiency mapping, we further narrowed down the *spe-43* genetic region. Male *spe-43(eb63)* worms were mated in a 4:1 ratio with hermaphrodites from two different deficiency lines: *sDf21*and *sDf22*. Heterozygous broods from the different crosses were isolated and scored for the *spe-43* infertility phenotype. *sDf21*and *sDf22* failed to complement *spe-43*, consistent with other mapping data.

2.9. Whole genome sequencing

spe-43 hermaphrodites were crossed with males from the polymorphic wild-type Hawaiian strain CB4856. F_1 heterozygous hermaphrodites were crossed with sibling F_1 heterozygous males in a ratio of 3:15. Individual F_2 hermaphrodites were picked at the L4 stage to 15