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The genetic and molecular analysis of *spe-19*, a gene required for sperm activation in *Caenorhabditis elegans*

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Abstract

During the process of spermiogenesis (sperm activation) in *Caenorhabditis elegans*, the dramatic morphological events that ultimately transform round sessile spermatids into polar motile spermatozoa occur without the synthesis of any new gene products. Previous studies have identified four genes (*spe-8*, *spe-12*, *spe-27* and *spe-29*) that specifically block spermiogenesis and lead to hermaphrodite-specific fertility defects. Here, we report the cloning and characterization of a new component of the sperm activation pathway, *spe-19*, that is required for fertility in hermaphrodites. *spe-19* is predicted to encode a novel single-pass transmembrane protein. The *spe-19* mutant phenotype, genetic interactions and the molecular nature of the gene product suggest SPE-19 to be a candidate for the receptor/co-receptor necessary for the transduction of the activation signal across the sperm plasma membrane.

Keywords: Fertilization; Sperm; Spermiogenesis; Spermatogenesis; *C. elegans*

Introduction

Differentiating cells typically undergo dramatic morphogenetic changes to form and maintain structures crucial for the initiation and maintenance of that differentiation. For most studied cell types, this usually involves the synthesis of new and specialized gene products (Blau, 1992; Chandrasekhar et al., 1992; Finney et al., 1987; Kraft et al., 1989; Shapiro, 1985; Simon et al., 1991; Simons and Fuller, 1985; Simons and Wandinger-Ness, 1990). In some cell types, however, morphological change is known to occur in the absence of any new macromolecular synthesis. Anucleate platelets, for example, differentiate rapidly during blood clotting, accomplished primarily via actin cytoskeleton alterations (Derenlau, 1987; Nachmias et al., 1987). Egg cortical granule fusion via calcium-calmodulin signaling and the sperm acrosome reaction serve as other well-known examples (Primakoff and Myles, 2002; Vacquier, 1975; Wassarman et al., 2001). The nematode *Caenorhabditis elegans* serves as an ideal model organism to examine cellular differentiation and morphogenesis via post-translational modification, as *C. elegans* sperm synthesize no new mRNA or protein (indeed, they contain no ribosomes), yet complete a remarkable morphological transformation from spermatid to spermatozoa (Geldziler et al., 2004; L'Hernault, 1997; L'Hernault and Singson, 2000; Singson, 2001).

Spermiogenesis in *C. elegans* is a rapid process, typically taking less than 5 min to complete (Nelson and Ward, 1980). Although its onset differs between males and hermaphrodites (hermaphrodite spermatids undergo activation after entry into the spermatheca, while males undergo activation soon after ejaculation into the uterus), the events are similar regardless of sperm derivation. Upon exposure to an unidentified activation signal, round sessile spermatids begin to polymerize filaments of Major Sperm Protein (MSP), which then coalesce to form the dynamic cytoskeleton and polar pseudopod needed by the sperm for motility

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and thus fertilization competence. On the opposite side of the cell, novel structures called membranous organelles (MO) fuse with the plasma membrane (Achanzar and Ward, 1997; L'Hernault, 1997; Shakes and Ward, 1989a; Singson, 2001). The function of these MOs and their fusion is not known.

Mutations in four genes, *spe-8*, *spe-12*, *spe-27* and *spe-29*, specifically affect this process of spermiogenesis in hermaphrodites, leading to a hermaphrodite-specific fertility defect in these animals. Available genetic and phenotypic evidence suggests that *spe-8*, *spe-12*, *spe-27*, *spe-29* and *spe-6* act in a common pathway (Muhlrad and Ward, 2002; Nance et al., 1999, 2000). Here, we present a phenotypic, genetic and molecular analysis of a new gene, *spe-19*, that is required for spermiogenesis in hermaphrodites but not males in vivo and whose mutants share the general *spe-8* class phenotype. We have cloned *spe-19*, which is predicted to encode a novel 300 amino acid type 1 transmembrane protein.

Materials and methods

Worm culture and strains

All strains used were maintained on *Escherichia coli OP50*-seeded NGM plates and manipulated as previously described (Brenner, 1974). The *C. elegans* Bristol strain N2 is considered wild-type, and the following mutants were also used in this study: *spe-8(hc53) dpy-5(e61)*, *spe-6(hc163), spe-8(hc40), dpy-18(e364)*, *dpy-21(e428)*, *unc-76(e911)*, *unc-51(e1189)*, *rol-9(sc149)*, *him-5(e1490), dpy-5(e61)*, *fog-2(q71)*, *fer-1(hc1ts)*, *fem-1(hc17)*, ozDF1/*sdc-3(y52y180), unc-76(e911)*V, ozDF2/*dpy-21(e428), par-4(it33)*V, yDF4/*dpy-11(e224)*, *unc-76(eq11)*V. The Hawaiian strain CB4856 was used for SNP mapping. *spe-19(hc41)* was isolated from a sperm development screen in the Ward laboratory (Shakes, 1988), and *spe-19(eb52)* was isolated from an ethylmethanosulfate (EMS) mutagenesis conducted by Mako Saito and Tim Schedl. *spe-6(h163)* worms were provided by Paul Muhlrad and Sam Ward. All other strains were provided by the CGC. Descriptions of all mutants used in this study may be found in Hodgkin (1997).

Worm genetics

spe-19 was mapped using a combination of standard two-point, three-point, deficiency and snip-SNP techniques (Jakubowski and Kornfeld, 1999). *spe-19* lies on the far right of Chromosome V (Fig. 6A). Three-point mapping using *dpy-21* and *rol-9* gave two recombinant classes, suggesting that *spe-19* lies between the two genes and closer to *rol-9* (Rol non-Dpy: 5/132 Spe, Dpy non-Rol 4/13 Spe).

Two-point data using *dpy-21 spe-19* recombinants generated from the above three-point mapping experiment suggested that *spe-19* lies approximately 11.4 MU to the right of *dpy-21* at approximately position 24.6 (16/70 Dpy Spe).

Of the three deficiencies in the *spe-19* region, *spe-19(eb52)* and *spe-19(hc41)* complement yDF4(V) and fail to complement ozDF1(V), consistent with the above mapping data (Fig. 6A). Importantly, *spe-19*/ozDF1 worms exhibit no additional phenotypes, suggesting that sterility is the null phenotype. Interestingly, *spe-19* also complemented ozDF2(V) whose predicted right end lies at map position 25.12, suggesting that perhaps this deficiency is complex.

unc-76; spe-19 and *spe-19; rol-9* double mutants were created and used for snip-SNP mapping. These worms were first crossed into Hawaiian strain CB4856, and their progeny allowed to self-fertilize. Recombinants were then picked and assayed for the presence of single nucleotide polymorphisms. Snip-SNP mapping using these *unc-76* non-*spe-19* (n = 48) and *rol-9* non-*spe-19* (n = 6) recombinants enabled us to map *spe-19* to the ~450 kb interval between cosmids M162 (map position 23.31) and ZC15 (map position 24.95), an interval predicted to contain approximately 100 genes on the far right of chromosome V. A search for candidate genes within the interval suggested Y113G7A.10 as a likely prospect as it is one of two genes in the region whose expression is sperm-enriched (Reinke et al., 2004).

Male lines were generated by first crossing *spe-19/+* males to *spe-19/spe-19* hermaphrodites. Single male progeny were then crossed to *spe-19/spe-19* hermaphrodites, L4 progeny were picked and scored for sterility. *spe-6* suppression experiments were performed as described by Muhlrad and Ward (2002) using the weak hypomorphic allele *spe-6(hc163)*. Homozygous *spe-6, dpy-18* hermaphrodites were mated to either homozygous *spe-19* or *fog-2* (a non-spermiogenesis hermaphrodite-specific sterile control) males. F1 heterozygotes were plated and allowed to self-fertilize. Dpy F2s were then plated and scored for sterility. Approximately one fourth of all Dpy progeny would be expected to be sterile unless this phenotype was suppressed by *spe-6(hc163)*. 0/315 Dpys were sterile when crossed to *spe-19* animals, while 44/179 Dpys were sterile using the *fog-2* control. To ensure that the homozygous *spe-19* class of animals was present, individual Dpy hermaphrodites were then crossed to homozygous *spe-19* males, and progeny were scored for sterility. Animals for whom all progeny were sterile (2 of 16) indicated that the *spe-19* homozygous class of animal was present.

Statistical analyses

Appropriate statistical tests for significance (t test, Mann–Whitney Rank Sum, ANOVA) were performed using SigmaStat (Systat Software, Inc. Point Richmond, CA).