spe-29 Encodes a Small Predicted Membrane Protein Required for the Initiation of Sperm Activation in *Caenorhabditis elegans*

Jeremy Nance,[1] Elizabeth B. Davis and Samuel Ward

Department of Molecular and Cellular Biology, University of Arizona, Tucson, Arizona 85721

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ABSTRACT

Caenorhabditis elegans spermatids complete a dramatic morphogenesis to crawling spermatozoa in the absence of an actin- or tubulin-based cytoskeleton and without synthesizing new gene products. Mutations in three genes (*spe-8*, *spe-12*, and *spe-27*) prevent the initiation of this morphogenesis, termed activation. Males with mutations in any of these genes are fertile. By contrast, mutant hermaphrodites are self-sterile when unmated due to a failure in spermatid activation. Intriguingly, mutant hermaphrodites form functional spermatozoa and become self-fertile upon mating, suggesting that spermatids can be activated by male seminal fluid. Here we describe a mutation in a fourth gene, *spe-29*, which mimics the phenotype of *spe-8*, *spe-12*, and *spe-27* mutants. *spe-29* sperm are defective in the initiation of hermaphrodite sperm activation, yet they maintain the ability to complete the morphogenetic rearrangements that follow. Mutant alleles of *spe-12*, *spe-27*, and *spe-29* exhibit genetic interactions that suggest that the wild-type products of these genes function in a common signaling pathway to initiate sperm activation. We have identified the *spe-29* gene, which is expressed specifically in the sperm-producing germ line and is predicted to encode a small, novel transmembrane protein.

CELLS acquiring specialized functions often undergo morphogenetic rearrangements to form structures crucial for their performance. Though our understanding of how these structural changes are regulated is incomplete, mechanisms controlling cellular morphology have been described in a few well-studied cell types and in many cases have been conserved. For example, the polarized morphogenesis that precedes mating in the budding yeast *Saccharomyces cerevisiae* initiates when haploid cells respond to a mating pheromone secreted by neighboring cells of the opposite mating type. Mating pheromone activates a mitogen-activated protein (MAP) kinase signaling cascade and the small GTPase Cdc42p, which ultimately results in modification of the actin cytoskeleton and cell wall (reviewed in Leberer *et al.* 1997; Madden and Snyder 1998). In fibroblasts, members of the MAP kinase signaling cascade and the small GTPases Cdc42 and Rac function to regulate morphological responses to growth factors (reviewed in Kyriakis and Avruch 1996). Cdc42 and Rac are also thought to mediate cytoskeletal changes as anucleate platelets activate during blood clotting (Azim *et al.* 2000). In these well-studied examples, morphogenetic change is largely accomplished through alteration of the actin cytoskeleton.

Corresponding author: Samuel Ward, MCB Department, University of Arizona, Life Sciences South No. 452, Tucson, AZ 85721. E-mail: samward@u.arizona.edu

[1] *Present address:* Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109-1024.

Spermatids from the nematode *Caenorhabditis elegans* undergo a dramatic and sudden morphogenesis during their activation to form crawling spermatozoa and thus provide an ideal model for examining cellular morphogenesis in a genetically tractable metazoan. Activation of self-sperm begins within the hermaphrodite when spermatids are first pushed into the spermatheca by oocytes and perceive an unidentified endogenous activator (Ward and Carrel 1979). Spermatids from males activate soon after they are ejaculated into the hermaphrodite uterus. Within minutes, membranous organelles fuse with the spermatid plasma membrane, a polar pseudopod extends, and the major sperm protein polymerizes and organizes into a pseudopodial cytoskeleton (Nelson and Ward 1980). All of these changes are needed for spermatozoa to crawl and fertilize oocytes. Remarkably, these rearrangements occur without regulation of a classical actin- or tubulin-based cytoskeleton (spermatids have neither) and in the absence of new gene product synthesis (spermatids have no ribosomes; Ward *et al.* 1981; Ward 1986; Pavalko and Roberts 1989). Because of their unusual cell biology, nematode sperm may utilize unconventional mechanisms to regulate their morphogenesis to spermatozoa. Several mutations, identified through screens for genes required for sperm development, specifically impair the initiation of spermatid activation and cause nearly identical phenotypes: spermatids from *spe-8*, *spe-12*, or *spe-27* mutants fail to activate in virgin hermaphrodites, yet both self-spermatids and male-derived spermatids activate normally in mated hermaphrodites. The phenotype of these

mutants predicts that males and hermaphrodites provide functionally distinct signals that can activate spermatids from either sex (Shakes and Ward 1989; Minniti *et al.* 1996; Nance *et al.* 1999).

By continued investigation of this signaling pathway, we hope to understand the control of morphogenesis in a cell lacking both a conventional cytoskeleton and a means of synthesizing new gene products. Here we describe a fourth gene, *spe-29*, which is required specifically for spermatids to initiate activation. *spe-29* mutants display the same characteristic activation defects observed in *spe-8*, *spe-12*, and *spe-27* mutants. We demonstrate that *spe-29*(*it127*) and mutant alleles of other members of this pathway interact genetically. We have cloned the *spe-29* gene, which is expressed during spermatogenesis and is predicted to encode an unusually small transmembrane protein.

MATERIALS AND METHODS

Worm culture and genetics: Worms were cultured and crossed as described by Brenner (1974). The following mutant alleles, derivatives of the wild-type N2 (var. Bristol) strain, were utilized in this study: LGI: *unc-13*(*e51*) (Brenner 1974), *spe-12*(*hc76*) (L'Hernault *et al.* 1988), and *fer-1*(*hc13*ts, *b232*ts) (Ward and Miwa 1978); LGIV: *fem-1*(*hc17*ts) [referred to as *fem-1*(1f)] (Nelson *et al.* 1978), *spe-27*(*it132*ts) (Minniti *et al.* 1996), *unc-24*(*e138*) (Brenner 1974), *elt-1*(*zu180*) (Page *et al.* 1997), *egl-20*(*n585*) (Maloof *et al.* 1999), *eDf19*, *fem-3*(*q23*ts) [referred to as *fem-3*(gf)] (Barton *et al.* 1987), *mDf7*, *spe-29*(*it127*) (this study), *egl-38*(*s1775*) (Chamberlin *et al.* 1997), and *dpy-20*(*e1282*ts) (Hosono *et al.* 1982); LGV: *him-5*(*e1490*) (Hodgkin *et al.* 1979). The *pKP614* Tc1 transposable element insertion is present in the Bergerac-derived strain RW7000, version NL (Korswagen *et al.* 1996).

it127 was positioned between *egl-20* and *pKP614* using a combination of deficiency and recombinational mapping techniques; results from all mapping experiments are accessible online in the Wormbase database (http://www.wormbase.org). When mapping *it127* with respect to *pKP614*, individual recombinants were scored by PCR (Williams *et al.* 1992; Korswagen *et al.* 1996) using a Tc1-specific oligonucleotide (TCACAAGCTGATCGACTCGATGC) and a flanking genomic oligonucleotide (ACGAAAGGACACTAACGGGCGG). Since *it127* mapped near no well-positioned *spe* genes and complemented several poorly characterized alleles that result in a Spe phenotype, it was assigned the new gene designation *spe-29*.

spe double mutants were constructed using separate strategies for unlinked and linked mutants. Unlinked *spe* mutations were combined by generating a doubly heterozygous F_1 where each *spe* (+) chromosome was marked with a recessive morphological marker mapping to a similar genetic position; F_2 were then screened for individuals that carried neither marker. Using this strategy, *spe-12* and *spe-27* were combined by mating *spe-12*; *dpy-20*; *him-5* males to *unc-13*; *spe-27*; *him-5* hermaphrodites. *spe-12* and *spe-29* were similarly combined by mating *spe-12*; *dpy-20*; *him-5* males to *unc-13*; *spe-29*; *him-5* hermaphrodites.

The linked mutations *spe-27* and *spe-29* were combined by utilizing morphological markers to identify chromosomes that had recombined between the two loci (which are 2 cM distant). *spe-27 dpy-20*; *him-5* males were mated to *unc-24 spe-29*; *him-5* hermaphrodites. Outcross males (non-Unc) were mated to *unc-24 spe-29 dpy-20*; *him-5* hermaphrodites. The *spe-27 spe-29* double mutant was isolated from a non-Unc non-Dpy Spe child of this cross.

In all cases, the genotype of double mutants was verified by complementation analysis using multiple hermaphrodites from each strain. Since double-mutant males were fertile, strains could be maintained unbalanced. The *him-5*(*e1490*) mutation was included in each double mutant strain to aid in the initial isolation of males.

Worm synchronization and progeny counts: Synchronized virgin hermaphrodites were obtained by allowing gravid hermaphrodites to lay eggs on a seeded plate for 5 hr. When males were present in the brood, hermaphrodites were separated from males during the final larval stage (L4) to preserve their virginity.

To determine self-brood sizes, hermaphrodites were individually picked at the L4 stage to seeded plates and allowed to self-fertilize at the indicated temperature. Gravid hermaphrodites were transferred to new plates daily until no new eggs were laid in a 24-hr interval. Progeny were recorded as they were removed by aspiration. If hermaphrodites were nearly sterile (Spe mutants), F_1 were recorded and removed from the plate before reaching sexual maturity. When *him-5* hermaphrodites were utilized, the progeny number reflected the sum of dead eggs (a result of the *him-5* mutation) and live offspring. Hermaphrodites that died prematurely (while still holding fertilized eggs) were excluded from the analysis. All errors presented are the standard error of the mean (SEM).

Transactivation assays: Sperm from Spe hermaphrodites were transactivated by mating synchronized virgin hermaphrodites with three sterile *fer-1* males for a 24-hr interval. Animals were placed together when hermaphrodites were at the L4 stage; males were at the L4 or young adult stage. *fer-1*(*hc13*ts); *him-5* males were utilized in assays that were conducted at 25°, while *fer-1*(*b232*ts); *him-5* males (which are sterile at 20°) were utilized in assays that were conducted at 20°.

Sperm counts: Synchronized virgin hermaphrodites grown at 25° were picked at the L4 stage to individual plates. After maturing to adulthood and laying eggs or oocytes for 3–5 hr, each was fixed and stained with DAPI (4′,6-diamidino-2-phenylindole) and the sperm nuclei present in the spermathecae and uterus were recorded as described by Nance *et al.* (1999). For *him-5* controls, the number of fertilized eggs laid before fixation was added to the sperm count. *him-5* hermaphrodites laid 45 ± 8.8 (SEM) eggs before fixation, *spe-29* hermaphrodites laid 23 ± 1.3 oocytes before fixation, and *spe-12* hermaphrodites laid 24 ± 2.3 oocytes before fixation.

Transformation of *spe-29* hermaphrodites: Adult hermaphrodites were transformed with cosmid or plasmid DNA (at 20 ng/μl) as described by Mello and Fire (1995). pRF4, which contains the dominant *rol-6*(*su1006*) allele, was coinjected (at 100 ng/μl) to facilitate recognition of transformed animals (which roll; Kramer *et al.* 1990). After recovery, each injectee was mated overnight with four *spe-29* males. Transformed hermaphrodite F_1 progeny of injected worms were picked to individual plates as virgins and scored for self-fertility. Fertility was always assessed in the F_1 generation to minimize germ-line silencing effects on the transformed DNA (Kelly *et al.* 1997; Kelly and Fire 1998).

Construction of wild-type and mutated genomic subclones of cosmid F25H8: Standard techniques were used to isolate and manipulate DNA (Ausubel *et al.* 1995). When PCR-amplified DNA was cloned, all relevant coding regions were sequenced in their entirety to ensure that no mutations were introduced. All oligonucleotide sequences are presented in the 5′ to 3′ orientation.

Unmodified subclones of F25H8 were obtained by cloning various restriction fragments into the pBluescript II SK^+ vector

(Stratagene, La Jolla, CA). The pJN115 subclone was constructed by purifying the 4488-bp *Bgl*II-to-*Xba*I fragment of F25H8 and ligating it into pBluescript II SK$^+$ cut with *Bam*HI and *Xba*I.

Plasmid pJN136 was constructed from pJN115 by an inverse PCR-based site-directed mutagenesis procedure. Briefly, oligonucleotides were designed from opposite strands such that their 5′ bases were abutting, save an additional base that was added to the 5′ end of one oligonucleotide (**C**AAATCTGGAT GATTCAATTGTATG; TCGACTTTAATTGTTTCTTCCTCC; boldfaced **C** is the added base). Using these oligonucleotides and pJN115 as template, a linear version of pJN115 with an additional nucleotide at one terminus was amplified. Purified PCR product was phosphorylated, circularized, and transformed into bacteria for recovery. The inserted base pair is predicted to result in a frameshift early in the first exon of F25H8.2, which would terminate translation after the 12th codon.

Plasmid pJN142 was constructed from pJN137, a version of pJN115 mutated by the above procedure to introduce a *Spe*I site early in the *spe-29*-translated region (first four codons of *spe-29* in pJN137: ATGACGACTAGT; *Spe*I site is underlined). To create pJN142, pJN137 was cleaved with *Spe*I, sticky ends were filled with Klenow, and the linear plasmid was circularized. The Klenow fill creates a 4-bp insertion that disrupts the reading frame and is predicted to terminate translation of *spe-29* mRNA after the fourth codon.

Isolation of *spe-29* cDNAs and identification of the *it127* lesion: *spe-29* cDNAs were isolated by PCR from a *fem-3*(gf) cDNA library directionally cloned into a pBluescript-derived plasmid vector (library generously provided by H. Smith). To amplify the 5′ end of *spe-29*, PCR reactions were performed with a vector-specific oligonucleotide (ACGTTGTAAAACG ACGGCCAGTGA) and a *spe-29* oligonucleotide from exon II (GACTCCGACGAGAAGAACTGAG). The 3′ end of the *spe-29* transcript was amplified with an oligonucleotide from exon I (CCGAATTTGGTTCATCTGCAGCTG) and a vector-specific oligonucleotide (GAAACAGCTATGACCATGATTACGC). PCR products were cloned into the pCR2.1 vector (Invitrogen, San Diego). Three clones from the 5′ end of the gene and three clones from the 3′ end of the gene (which partially overlapped the 5′ end clones) were sequenced.

The *spe-29*(*it127*) lesion was identified by comparing the sequences of genomic DNA amplified by PCR from individual wild-type and *spe-29* worms (Williams 1995). Oligonucleotides CCGAATTTGGTTCATCTGCAGCTG and CATTCCCT CAATCACCTAACACTAGG were used in the amplification and sequencing reactions. A single G-to-A base change was detected in this interval. To ensure that this mutation was present in the genomic template and was not established during amplification, new genomic DNA was amplified from *spe-29* worms and sequenced. All sequencing reactions were performed by the University of Arizona Laboratory of Molecular Systematics and Evolution sequencing facility using an ABI Prism sequencer (Applied Biosystems, Foster City, CA) and the manufacturer's protocol.

Northern analysis: RNA was isolated from synchronized *fem-1*(1f) and *fem-3*(gf) adults aged 68–70 hr (25°) as described by Nance *et al.* (1999). A total of 4 μg of poly(A)-enriched RNA from each mutant was analyzed by Northern analysis as described in Ausubel (1995). Blots were probed with a PCR-amplified full-length *spe-29* cDNA (see above).

RESULTS

***spe-29* spermatids fail to activate to spermatozoa:** Among a collection of previously uncharacterized spermatogenesis-defective mutants, we identified one, *it127*, with defects in spermatogenesis nearly identical to those found in *spe-8*, *spe-12*, and *spe-27* mutants. We positioned *it127* on LGIV in a region that defines it as a new *spe* gene, which we designated *spe-29* (see materials and methods). *it127* is the only mutant allele of *spe-29*. We abandoned efforts to identify additional alleles of *spe-29* after realizing that the gene was unusually small and was closely abutted by flanking genes, so it provided an exceptionally small target for random mutagenesis (see molecular characterization below).

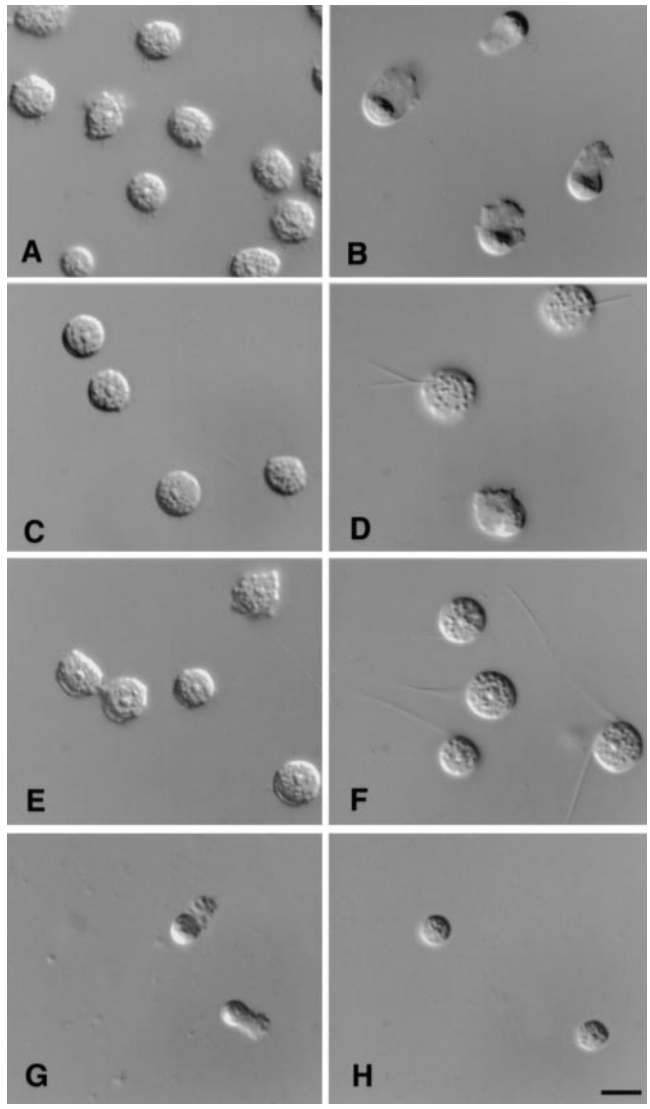

Figure 1.—Morphology of wild-type and mutant sperm activated *in vitro* and *in vivo*. (A–F) Spermatids from adult males were dissected in either sperm medium (A, C, and E) or in sperm medium containing protease (pronase; B, D, and F), as described in Shakes *et al.* (1989). (A and B), wild type; (C and D), *spe-29*; (E and F), *spe-12*. Sperm shown in G and H were dissected from young adult hermaphrodites into sperm medium. (G) Wild type; (H) *spe-29*.

In vitro, when treated with proteases that prompt wild-type spermatids to form pseudopods (Figure 1, A and

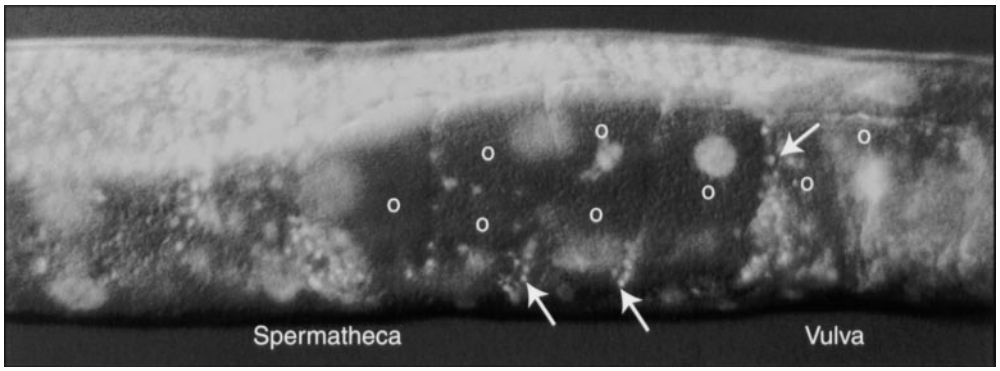

FIGURE 2.—Spermatids in the uterus of a *spe-29* hermaphrodite. A young adult *spe-29* hermaphrodite that had just begun to lay oocytes was fixed and nuclei were stained as described in MATERIALS AND METHODS. Only one-half of the reproductive tract is shown. The spermatheca and vulva are labeled below. Each oocyte (o) in the uterus is indicated. Sperm, recognized by their compact nuclei, are visible in the spermatheca and sandwiched between oocytes in the uterus (examples are indicated with arrows).

B), most *spe-29* spermatids arrested their morphogenesis after extending spiky projections, although a few formed apparently normal pseudopods (Figure 1, C and D). Sperm from *spe-12* (as well as *spe-8* and *spe-27*) mutants extend identical spiky projections (Figure 1, E and F; SHAKES and WARD 1989). Also, like the previous mutants, *spe-29* spermatids treated with triethanolamine, which increases intracellular pH, activated normally and formed motile pseudopods (data not shown).

Spermatids within *spe-29* virgin hermaphrodites are unable to activate, mimicking the phenotype of *spe-8*, *spe-12*, and *spe-27* sperm. Although mutant hermaphrodites produce many spermatids (see below), these spermatids rarely activate to form normal crawling spermatozoa (Figure 1, G and H). Consequently, hermaphrodites are unable to self-fertilize their oocytes. Many of these immotile spermatids are dislodged from the spermatheca and swept away as oocytes parade through the reproductive tract (Figure 2). Some spermatids (~50 per spermatheca; see below) manage to avoid being dislodged and are retained in the spermatheca.

Self-sterility in *spe-29* mutants is not absolute and is somewhat temperature sensitive: virgin hermaphrodites lay only occasional self-fertilized eggs at 15° and 25°, but produce more at 20° (6.9 ± 1.2 progeny, $n = 15$; wild type produced 257 ± 13.6 progeny at 20°, $n = 10$). In wild type and many mutants with a leaky Spe phenotype, oocytes are self-fertilized nearly continuously until the supply of spermatozoa is exhausted (WARD and CARREL 1979; L'HERNAULT *et al.* 1988). By contrast, *spe-29* hermaphrodites lay infrequent self-fertilized eggs and many unfertilized oocytes for up to 5 days after the onset of ovulation, suggesting that a few *spe-29* spermatids activate sporadically well after ovulation begins (data not shown). These eggs develop into Spe hermaphrodites with no other obvious developmental defects.

***spe-29* self-spermatids can be efficiently rescued by mating:** *spe-29* spermatids could fail to initiate activation because of defects in signaling. Alternatively, *spe-29* spermatids may initiate but fail to complete activation because of defects in the cellular machinery required for morphogenesis. *spe-8*, *spe-12*, or *spe-27* sperm are defective specifically in the initiation of activation because these mutant spermatids are able to form functional spermatozoa when exposed to male seminal fluid after mating, a phenomenon we refer to as "transactivation." [*spe-8*, *spe-12*, and *spe-27* males are also fertile because their sperm are bathed in seminal fluid during ejaculation (SHAKES and WARD 1989; MINNITI *et al.* 1996; NANCE *et al.* 1999).] To test whether *spe-29* spermatids could be transactivated, we mated *spe-29 dpy-20* virgin hermaphrodites with sterile *fer-1*; *him-5* males, which function as donors of seminal fluid. Mated *spe-29 dpy-20* hermaphrodites produced self-brood sizes (recognized by their Dpy phenotype) that in some cases approached levels of wild-type (*dpy-20*) controls and were substantially higher than those obtained from virgin *spe-29 dpy-20* hermaphrodites (Table 1). The ability of *spe-29* spermatids to activate when exposed to a signal (which we refer to as male activator) supplied in the ejaculate indicates that they have all the components necessary to form functional spermatozoa but are defective in their response to an activation signal (which we refer to as hermaphrodite activator) present within virgin hermaphrodites.

***spe-29*(*it127*) and *spe-12*(*hc76*) are dominant enhancers of *spe-27*(*it132*):** The similarity in phenotype of *spe-8*,

TABLE 1

Transactivation of *spe-29* self-sperm

Hermaphrodite genotype	Average self-progeny	Range	Sample size
spe-29 dpy-20 (virgin)	2.4 ± 0.7	1–11	10
spe-29 dpy-20 (× *fer-1* male)	116 ± 10	26–166	14
dpy-20	166 ± 9.1	109–240	16

TABLE 2

Enhancement of Spe-27 self-sterility by *spe-29* and *spe-12*

Hermaphrodite genotype[a]	Self-progeny[b]	Sample size
spe-27	8 ± 1.2	22
spe-29	0.5 ± 0.1	20
spe-12	0.07 ± 0.07	15
spe-27 spe-29	0	15
spe-27; *spe-12*	0	15
spe-27 spe-29/*spe-27 spe-29*(+)	3.4 ± 0.6	25
spe-27; *spe-12*/*spe-12*(+)	1.4 ± 0.3	24
Wild type	274 ± 10.5	11
spe-29/*spe-29*(+)	282 ± 7.3	14
spe-12/*spe-12*(+)	292 ± 5.7	14

[a] All animals were also homozygous for the *him-5*(*e1490*) mutation. Wild type refers to *him-5*(*e1490*).

[b] Self-progeny includes the sum of live progeny and dead eggs (a consequence of the *him-5* mutation). All animals were selfed at 15°. Values are expressed as the average ± the SEM.

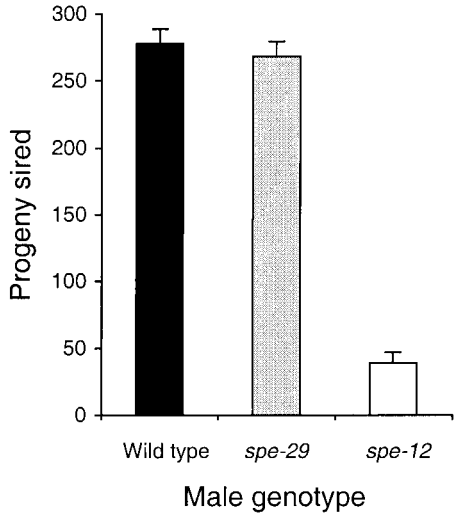

FIGURE 3.—*spe-29* male fertility. Single young adult males of the indicated genotype were placed with a single young adult *unc-24 dpy-20* hermaphrodite and allowed to mate for 24 hr. Outcross progeny (non-Unc non-Dpy) were counted. Wild type, $n = 21$; *spe-29*, $n = 23$; *spe-12*, $n = 12$. Error bars represent the SEM.

spe-12, *spe-27*, and *spe-29* mutants suggested that the products of these genes are needed specifically for the function of a signaling pathway required for spermatid activation. If so, this pathway might be compromised by manipulating the levels of these gene products in combinations by varying gene dosage. We looked for such genetic interactions among mutant alleles of these four genes (Table 2). Few *spe-27*(*it132*ts) self-spermatids activate and fertilize oocytes at the permissive temperature (Table 2; MINNITI *et al.* 1996). We added mutant alleles of the other activation genes to *spe-27* mutants and examined the self-fertility of the resulting worms. The phenotypes of *spe-27* hermaphrodites also homozygous for the nonconditional alleles *spe-29*(*it127*) or *spe-12*(*hc76*) were not informative, since each double mutant adopted the epistatic phenotype of the nonconditional allele. However, when *spe-27* hermaphrodites heterozygous for either *spe-29* or the null allele *spe-12*(*hc76*) were selfed, brood sizes were reduced significantly (MANOVA; $P < 0.001$). Both *spe-29*(*it127*) and *spe-12*(*hc76*) were completely recessive in an otherwise wild-type background. Thus *spe-29*(*it127*) and *spe-12*(*hc76*), while normally recessive, are each dominant enhancers of *spe-27*(*it132*ts) when sperm are activated in virgin hermaphrodites, consistent with the products of these genes functioning in a common signaling pathway.

***spe-29* spermatids are normal in their response to male activator:** In null alleles of *spe-12*, such as *hc76*, transactivation of self-spermatids by mating is never efficient, rescuing only a small fraction of spermatids present within hermaphrodites at the time of mating (NANCE *et al.* 1999). When we transactivated *spe-29* spermatids, we noticed that hermaphrodites produced many more self-progeny than typically observed when *spe-12* spermatids are transactivated. To confirm that the efficiency of

TABLE 3

Transactivation efficiency and retention of *spe-29* and *spe-12* sperm

Hermaphrodite genotype[a]	Self-progeny[b]	Range	*n*[c]	Sperm[d]
spe-29	0.7 ± 0.4	1–11	10	88 ± 5.4
spe-12	0.2 ± 0.1	0–1	10	97 ± 13
spe-29 (× *fer-1* male)	82 ± 13	14–166	14	NA
spe-12 (× *fer-1* male)	3.4 ± 1.2	0–16	15	NA
Wild type	110 ± 11	43–162	10	137 ± 14

[a] All animals were also homozygous for the *him-5*(*e1490*) mutation. Wild type refers to *him-5*(*e1490*).

[b] Self-progeny includes the sum of live progeny and dead eggs (a consequence of the *him-5* mutation). All animals were selfed at 25°. Values are expressed as the average ± the SEM.

[c] *n*, sample sizes for worms used to determine brood sizes.

[d] Separate virgin hermaphrodites were used to determine the average number of sperm in each strain (*him-5*, $n = 5$; *spe-29*; *him-5*, $n = 15$; *spe-12*; *him-5*, $n = 11$; see MATERIALS AND METHODS).

transactivation of *spe-12* and *spe-29* spermatids differed significantly, we compared the self-brood sizes of mated *spe-12* and *spe-29* hermaphrodites to the number of spermatids present in control unmated mutants of equivalent age. As shown in Table 3, mating restores the self-fertility of *spe-29* hermaphrodites to a much greater extent than it does for *spe-12*. Two observations suggest that most or all spermatids present within a mated *spe-29* hermaphrodite activate to spermatozoa. First, broods from some *spe-29* hermaphrodites mated to *fer-1* males were as large as brood sizes from unmated wild-type controls (Table 3). Second, the average brood size of transactivated *spe-29* hermaphrodites (82) nearly matched the average number of sperm present within similarly aged virgin *spe-29* hermaphrodites (88). Only a few self-progeny were produced by mated *spe-12* hermaphrodites even though *spe-12* hermaphrodites had as many sperm as *spe-29* hermaphrodites, indicating that *spe-29* sperm are transactivated more efficiently than *spe-12* sperm.

We also tested the ability of *spe-29*-male-derived sperm to activate by assessing the fertility of individual *spe-29* males. *spe-29* males were as fertile as wild-type males; in comparison, fertility of *spe-12* males was significantly impaired (Figure 3) even though both *spe-12* and *spe-29* males produce seemingly normal numbers of sperm (data not shown) and *spe-12* males copulate normally (Nance *et al.* 1999). Thus, while *spe-29* spermatids fail to activate in response to hermaphrodite activator alone, unlike *spe-12* mutants they are wild type in response to male activator.

***spe-29*(*it127*) impairs response to male activator in a sensitized genetic background:** *spe-12* sperm are defective in their response to both hermaphrodite and male activator, while *spe-29* sperm are defective only in their response to hermaphrodite activator. We reasoned that if *spe-29*(+) is not needed for response to male activator, then the efficiency of transactivation of another activation mutant should not be influenced by the *spe-29* genotype. To test this hypothesis, we compared the self-fertility of *spe-27*(*it132*ts) hermaphrodites to *spe-27*(*it132*ts) *spe-29* hermaphrodites after each had been mated to *fer-1* males. At 20°, mated *spe-27* hermaphrodites had a Spe-29-like phenotype, producing many more self-progeny than mated *spe-12* null mutants (Table 4). However, when *spe-27 spe-29* double mutants were mated, self-brood sizes were substantially smaller than those of mated *spe-27* single mutants. By contrast, mated *spe-12* and *spe-12*; *spe-29* hermaphrodites had equivalent brood sizes (Table 4). At a higher temperature (25°), mated *spe-27* hermaphrodites yielded only a few self-progeny (Table 4), similar to mated *spe-12* mutants (see Table 3). Mutating *spe-29* had little effect on the already poor activation of *spe-27* sperm at 25°, since mated *spe-27 spe-29* hermaphrodites produced broods similar in size to those of mated *spe-27* hermaphrodites. Thus *spe-29* can impair the ability of self-sperm to respond to male activator, but only when the activation signaling pathway is compromised [at 20° in a hypomorphic *spe-27*(*it132*ts) background] but not blocked [in a null *spe-12* background or a *spe-27*(*it132*ts) background at 25°]. These data suggest that *spe-29*(*it127*) limits, but does not abolish, the effectiveness of the sperm activation signaling pathway.

TABLE 4

Transactivation of *spe-29* spermatids in a sensitized genetic background

Hermaphrodite genotype[a]	Average self-progeny[b]	Sample size
spe-29 (virgin)	3.9 ± 0.7	18
spe-29 (× *fer-1* male)	214 ± 8.5	15
spe-12 (virgin)	0	14
spe-12 (× *fer-1* male)	60 ± 5.7	20
spe-12; *spe-29* (virgin)	0.1 ± 0.1	10
spe-12; *spe-29* (× *fer-1* male)	55 ± 7.8	16
spe-27 (virgin)	0.1 ± 0.1	15
spe-27 (× *fer-1* male)	196 ± 16	20
spe-27 spe-29 (virgin)	0	13
spe-27 spe-29 (× *fer-1* male)	50 ± 5.0	29
spe-27 (× *fer-1* male; 25°)	2.5 ± 0.4	49
spe-27 spe-29 (× *fer-1* male; 25°)	1.5 ± 0.3	49

[a] All animals were also homozygous for the *him-5*(*e1490*) mutation. Unless indicated, animals were incubated at 20°.

[b] Self-progeny includes the sum of live progeny and dead eggs (a consequence of the *him-5* mutation). Error presented is the SEM.

***spe-29* is predicted to encode a small, novel membrane protein:** Through a series of genetic mapping experiments, we positioned *spe-29* on LGIV within the overlap of deficiencies *eDf19* and *mDf7* and between *egl-20* and *pKP614* (see MATERIALS AND METHODS). We assayed genomic cosmid clones from this ~100-kb region for their ability to restore self-fertility to *spe-29* hermaphrodites when introduced into the germ line. One cosmid, F25H8, significantly increased the self-fertility of mutants (data not shown). The sequence from this cosmid and its predicted genes was available from the *C. elegans* Sequencing Consortium (1998). We used this information to design subclones that contained individual

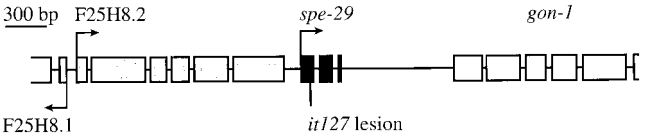

Figure 4.—Genes present on the pJN115-rescuing plasmid. pJN115 contains genes F25H8.1 (5′ portion only) and F25H8.2 (predicted by GENEFINDER), the 3′ end of *gon-1* (Blelloch and Kimble 1999), and *spe-29*. The 5′ end of each gene is marked with an arrow showing the direction of transcription. The location of the *it127* lesion within the *spe-29* gene is indicated with a line (see Figure 6).

TABLE 5

Transformation rescue of *spe-29* hermaphrodites

Construct	F25H8.2[a]	*spe-29*[a]	*n*[b]	Rescued lines[c]	Self-progeny[d]	Maximum progeny
—	—	—	40	NA	4	17
pJN115	+	+	64	7	80 ± 10	121
pJN136	—	+	72	13	81 ± 15	186
pJN142	+	—	123	0	NA	NA

[a] + or — refers to the presumed functionality of the indicated gene in each construct. See MATERIALS AND METHODS or details on gene disruption and construct synthesis.

[b] *n*, sample size of F_1 transformants (Rol phenotype).

[c] F_1 transformants were judged to be rescued if their F_2 brood sizes were ≥24 (2 standard deviations greater than the maximum number of progeny observed in untransformed animals) and contained some Rol individuals.

[d] Average self-progeny numbers were calculated only from rescued individuals, except for control injections, where broods from all F_1 transformants were averaged to determine the baseline fertility.

genes (when possible) and assayed these clones for their ability to restore self-fertility to *spe-29* hermaphrodites. Subclone pJN115, predicted to contain only one complete gene, F25H8.2 (Figure 4), was able to partially restore self-fertility to *spe-29* hermaphrodites (Table 5).

We compared the sequence of the F25H8.2 gene in wild-type and *spe-29*(*it127*) worms to identify the *it127* lesion. However, the sequence of wild type and *spe-29* were identical within F25H8.2, suggesting that F25H8.2 is not *spe-29*. We detected a point mutation in the predicted intergenic space (~1300 bp) between F25H8.2 and neighboring gene *gon-1* (Figure 4). When we probed a differential Northern blot with genomic DNA from this interval, we detected a single small transcript (~400 bp) in RNA from *fem-3*(gf) mutants (which make sperm but not oocytes) but not in RNA from *fem-1*(1f) mutants (which make oocytes but no sperm), indicating that a previously unidentified gene, which is expressed specifically in the sperm-producing germ line, resided in this small region (data not shown).

We searched for transcribed sperm-specific genes in this genomic interval by identifying oligonucleotides that could amplify a small cDNA from a *fem-3* (sperm) cDNA library but not a *fem-1* (oocyte) cDNA library. By utilizing this strategy, we pieced together a full-length cDNA that incorporated the *it127* lesion present in *spe-29* mutants (see MATERIALS AND METHODS). The sequence of this cDNA corresponded to a single small gene located entirely in the region between F25H8.2 and *gon-1* (Figure 4). Using probes from the full-length cDNA, we detected a single sperm-specific transcript equal in size to the originally observed transcript (~400 bp) and full-length cDNA (≥307 bp, see below; Figure 5).

To verify that *spe-29* was this small, previously unpredicted gene rather than the upstream gene F25H8, we created derivatives of the rescuing plasmid pJN115 that contained frameshift mutations in either F25H8.2 (pJN136) or *spe-29* (pJN142; see MATERIALS AND METHODS) and assayed their ability to restore self-fertility to *spe-29* hermaphrodites (Table 5). pJN136, which contained mutated F25H8.2 but wild-type *spe-29*, restored self-fertility to *spe-29* hermaphrodites as effectively as pJN115. However, pJN142, which contained wild-type F25H8.2 but mutated *spe-29*, failed to restore self-fertility to *spe-29* hermaphrodites. On the basis of on the identification of a missense mutation in this gene, its sperm-specific expression, and its ability to restore self-fertility to *spe-29* hermaphrodites, we conclude that it is indeed *spe-29*.

spe-29 is divided by three introns and potentially encodes a small, quite basic (pI = 9.6) peptide of 66 amino acids (Figure 6). While the predicted protein is novel, SPE-29 contains a strongly predicted transmembrane domain that occupies a full third of the protein; only

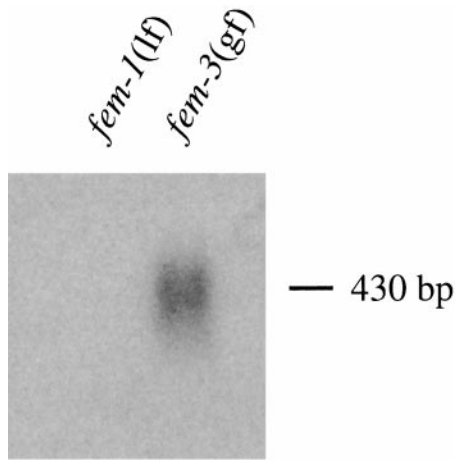

FIGURE 5.—Differential Northern analysis of the *spe-29* transcript. Poly(A)-purified RNA from *fem-1*(1f) mutants (somatically female, produce only oocytes) and *fem-3*(gf) mutants (somatically female, produce only sperm) was collected, separated, and probed with a full-length *spe-29* cDNA as described in MATERIALS AND METHODS. Amounts of *fem-1* and *fem-3* RNA were equivalent by ethidium bromide staining (data not shown).

```
aaaATGACGAATAGTCATACTCACAACGGAAACCGAATTTGGTTCATCTGCAGC  54
   M  T  N  S  H  T  H  N  G  N  R  I  W  F  I  C  S    17

                        (A:it127)
TGGGAAAATGGTCCAATTTGTTATGGAATGGCTCTgtgagttcaaattcttcgc 108
W  E  N  G  P  I  C  Y  G  M  A  L                      29
                        (E:it127)

agctgtgagctatgctctggaaatcgtatttttttcagTGATCTTATTGGGGGAA 162
                                      D  L  I  G  G  I   35

TTTCTTTGATTGCTTTCTCAGTTCTTCTCGTCGGAGTCATATTTTTCGGATGGT 216
  S  L  I  A  F  S  V  L  L  V  G  V  I  F  F  G  W  F  53

TTGGAATATTTCCAAAAGTTATTCGAgtaagcttcacgagtaattctaatttttt 270
  G  I  F  P  K  V  I  R                                61

gtatgttttctttcagCGTAAACTTCACGATTGAattttatatttttattataa 324
                R  K  L  H  D  stop                      66

cgttcagaagaaggcgaggctctattactctattttaataaaatatttgaattg 378

actaaagtttaataaatattatgcagtgcta                        409
```

FIGURE 6.—Wild-type and *it127* alleles of the *spe-29* gene and predicted translations. The nucleotide sequence shown begins with the first known base of the 5′ untranslated region (UTR) and ends with the last base of the 3′ UTR. Coding regions of the nucleotide sequence are in uppercase, while untranslated regions of the mRNA and introns are in lowercase. The predicted translation product is shown below the nucleotide sequence. The boldfaced nucleotide and amino acid are altered by the *it127* lesion; the corresponding *it127* sequence is shown above (nucleotide) and below (amino acid) the wild-type sequence. Amino acids within the predicted transmembrane domain are underlined. Nucleotides and amino acids (italics) are numbered on the right.

eight residues lie between the end of this domain and the carboxy terminus. The *it127* lesion results in a missense mutation (G26E) before the transmembrane domain.

DISCUSSION

SPE-29 is needed for sperm activation by both male and hermaphrodite activators: In severe alleles of *spe-8*, *spe-12*, and *spe-27*, spermatids do not activate at all in virgin hermaphrodites, while spermatids in mated animals (both self-sperm and male-derived sperm) activate with a lower efficiency than wild-type spermatids (NANCE *et al.* 1999). The unusual phenotype of these mutants suggests that the corresponding wild-type gene products function in an activation signaling pathway that responds to either a hermaphrodite signal or a male signal introduced by mating, which is presumably carried in the seminal fluid (SHAKES and WARD 1989; MINNITI *et al.* 1996; Figure 7). In this model, sperm could activate when supplied with either activator; the difference in phenotype of sterile hermaphrodites and fertile males could occur if hermaphrodite activator were less effective than male activator. Mutations in these genes would impair the signaling pathway such that weak hermaphrodite activator could not trigger

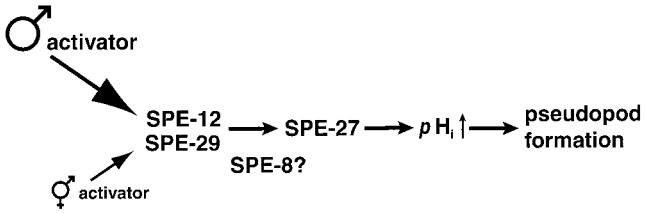

FIGURE 7.—Model for the initiation of sperm activation. Spermatids initiate activation upon receiving either of two activation signals: one that originates from within the hermaphrodite or one that is introduced by the male during mating. The signal, through the action of SPE-8, SPE-12, SPE-27, and SPE-29, likely induces an increase in pH_i. Morphogenetic rearrangements that lead to spermatozoon formation follow. SPE-12 (which is localized to the cell surface) and SPE-29 (which contains a transmembrane domain) are tentatively placed upstream of SPE-27 (a predicted cytoplasmic protein). In this model, absence of the SPE proteins would only impair the effectiveness of the pathway, such that weak hermaphrodite activator could not induce activation but more potent male activator could induce activation (albeit less effectively than in wild-type spermatids).

activation, but stronger male activator could induce at least some sperm to activate.

The Spe-29 phenotype suggests that SPE-29 function may be needed only when sperm utilize the hermaphrodite activator since self-sperm in virgin hermaphrodites do not activate, yet both male-derived and self-sperm in mated hermaphrodites activate normally. However, this phenotype could also arise if the *it127* mutation resulted in a partial loss of SPE-29 function sufficient to prevent activation via the hermaphrodite activator but retained enough activity to allow normal activation via the male activator. Several observations suggest that this is the case. First, unlike *spe-12* null mutants, *spe-29* hermaphrodites are slightly fertile, so some mutant sperm successfully activate even in virgin hermaphrodites. Second, some *spe-29* spermatids activate to spermatozoa when treated *in vitro* with proteases (this study) or the chloride channel blocker DIDS (MACHACA *et al.* 1996); this is in sharp contrast to *spe-12* sperm, which never activate normally when incubated with protease [SHAKES and WARD 1989; this study (Figure 1)] or DIDS (MACHACA *et al.* 1996). Third, while the Spe-29 phenotype is shared by some *spe-8* mutants (sperm activate efficiently when utilizing male activator), at least one *spe-8* allele results in a more severe Spe-12-like phenotype (sperm activate inefficiently when utilizing male activator; NANCE *et al.* 1999). These variations demonstrate that presumably hypomorphic alleles of a gene known to function in male activation cause less severe phenotypes than other, presumably null, alleles of this gene. Finally, the *it127* lesion is predicted to result in a single-amino-acid substitution, an alteration that could cause a partial loss of function of the mutant protein. Together, these observations suggest that *spe-29*(*it127*) is a hypomorphic allele and that *spe-29* null mutants would have the same pheno-

type as *spe-8*, *spe-12*, or *spe-27* null mutants. However, it remains possible that *spe-29*(*it127*) is a null allele and that *spe-29* plays a less critical role in sperm activation than do *spe-8*, *spe-12*, and *spe-27*.

Isolation of *spe-29* null mutants would be the best way to determine if *spe-29* is needed for sperm activation utilizing male activator, but this was not practical since the gene is exceptionally small and closely flanked by neighboring genes so that it provides an extremely small target for random mutagenesis. In addition, RNA-mediated interference with *spe-29* double-stranded RNA fails to produce any phenotype (as is true with nearly all *spe* genes assayed; data not shown; E. B. Davis, unpublished observations). Instead, we addressed this question by examining the effect of *spe-29*(*it127*) on sperm activation utilizing male activator in a sensitized genetic background. We found that *spe-27 spe-29* double-mutant spermatids were transactivated less efficiently than in either single mutant alone, indicating that *spe-29* is required for spermatids to activate efficiently in response to male activator.

SPE-12, SPE-27, and SPE-29 may function in a single signaling pathway: Despite the remarkable similarity in phenotype between *spe-8*, *spe-12*, *spe-27*, and *spe-29* mutants, we had no evidence that their encoded proteins were required for the function of a single signaling pathway. However, several genetic arguments described here support this hypothesis.

We have demonstrated that mutations in either *spe-12* or in *spe-29*, while completely recessive alone, function as dominant enhancers of the phenotype of a hypomorphic *spe-27* mutant in sperm that activates using hermaphrodite activator. Likely hypomorphic *spe-29* and *spe-27* mutations also act synergistically to reduce the efficiency of activation when sperm utilize male activator (discussed above). There are several interpretations of such genetic interactions. First, *spe-29* (and *spe-12*) could enhance the Spe-27 phenotype indirectly. This phenomenon has been observed in the fly eye, where genes such as *Notch*, *Star*, and *hedgehog*, which affect growth within the morphogenetic furrow of the developing eye disc, dominantly enhance the phenotype of *glass*^{3} mutants (Ma *et al.* 1996). The Glass transcription factor clearly functions well downstream of these genes in a distinct pathway that controls the terminal differentiation of eye photoreceptor cells. To avoid detecting such indirect genetic interactions, it is important to look for interactions among alleles of genes that are hypothesized to function toward the same endpoint. Since *spe-12*, *spe-27*, and *spe-29* all have remarkably similar phenotypes that implicate their involvement in transducing an activation signal, it is likely that enhancement of the Spe-27 phenotype by these mutants is direct.

Direct genetic interactions among mutants implies that the wild-type gene products function in the same biochemical pathway (Gu *et al.* 1996; Qu *et al.* 1999) or in partially redundant pathways (Thomas 1993). If two genes are required for the function of a single signaling pathway, then the phenotype resulting from a null mutation in one of these genes should not be worsened by loss-of-function mutations in the other. We observed that while *spe-29*(*it127*) dramatically lowers the self-fertility of mated hypomorphic *spe-27* mutants, *spe-29*(*it127*) does not reduce the self-fertility of mated *spe-12* null mutants or *spe-27* mutants grown at 25° (a temperature at which they behave as null mutants). These results are consistent with SPE-12, SPE-27, and SPE-29 functioning in a common sperm activation signaling pathway, though it remains possible that they act in separate pathways that lead to sperm activation.

Known null mutations in *spe-12* (as well as likely null mutations in *spe-8* and *spe-27*) completely block the activation of sperm in virgin hermaphrodites. However, mutant sperm that have been exposed to male seminal fluid are capable of activating, since *spe-12* males are somewhat fertile and since some self-sperm in a *spe-12* hermaphrodite can be transactivated. These observations suggest that the products of the activation genes may only have a supplementary role in the initiation of sperm activation, perhaps functioning to localize or stabilize effectors of the activation signal. Alternatively, male seminal fluid may contain an additional sperm activator that can initiate activation at a low level through a distinct pathway that does not require *spe-12* and the other activation genes.

Possible functional roles of SPE-29: Defective activation of *spe-29* spermatids *in vitro* (in protease) indicates that SPE-29 is not functioning as an exogenous activation signal (or a protein required for activator synthesis or delivery) and suggests that SPE-29 functions either to transduce the activation signal or properly localize or modulate proteins that are transducing this signal. The *spe-29* sequence reveals little about the likely molecular function of its product. SPE-29 is exceptionally small (a predicted 66 amino acids) and quite basic (pI = 9.6); its only feature of note is a strongly predicted internal transmembrane domain.

One interpretation of the observed genetic interactions among *spe-12, spe-29*, and *spe-27* alleles is that the wild-type gene products interact directly. Since SPE-12 is localized to the spermatid plasma membrane (Nance *et al.* 1999) and SPE-29 is predicted to be an integral membrane protein, one model for their function is that SPE-29 joins SPE-12 at the cell surface in a signaling complex that responds to the hermaphrodite and male activators (Figure 7). SPE-27, a predicted cytoplasmic protein, may function downstream of the membrane proteins (Minniti *et al.* 1996; Figure 7).

It is not known how these proteins mediate their signaling effects. One possibility, given that protease treatment of normal spermatids is sufficient to induce activation (Ward *et al.* 1983), is that SPE-12 and/or SPE-29 is proteolytically cleaved by an endogenous acti-

vator to commence the signaling cascade. These proteins are required at least indirectly for *in vitro* protease activation since *spe-8*, *spe-12*, *spe-27*, and *spe-29* spermatids activate defectively in proteases (Shakes and Ward 1989; Minniti *et al.* 1996; Figure 1).

The signaling pathway induces multiple changes in the spermatid leading to pseudopod formation. These include membrane rearrangements, membrane fusion, and assembly of the major sperm protein cytoskeleton to form the pseudopod. All of these processes can be induced simply by increasing the intracellular pH of spermatids with protoionophores or weak bases (Ward and Carrel 1979; Nelson and Ward 1980). Spermatozoa induced by pH increase appear normal and are capable of fertilization (Ward *et al.* 1983; LaMunyon and Ward 1994). pH is also known to be a vital element in the control of sperm activation in the nematode Ascaris; upon activation with purified male vas deferens extract, the pH of Ascaris spermatids rises and a stable pH gradient is established in the pseudopod where it is thought to directly regulate the assembly of major sperm protein fibers. SPE-8, SPE-12, SPE-27, and SPE-29 may act to increase intracellular pH since *spe-8*, *spe-12*, *spe-27*, and *spe-29* spermatids activate to form normal spermatozoa when their pH is increased *in vitro* with the weak base triethanolamine (Shakes and Ward 1989; Minniti *et al.* 1996; this study; Figure 7). Since a pH increase can bypass the entire activation signaling pathway it is likely that the endpoint of the pathway is regulation of intracellular pH, which would serve as a second messenger controlling the morphogenesis of spermatids.

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LITERATURE CITED

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman *et al.* (Editors), 1995 *Current Protocols in Molecular Biology*. John Wiley & Sons, New York.

Azim, A. C., K. Barkalow, J. Chou and J. H. Hartwig, 2000 Activation of the small GTPases, Rac and Cdc42, after ligation of the platelet PAR-1 receptor. Blood **95:** 959–964.

Barton, M. K., T. B. Schedl and J. Kimble, 1987 Gain-of-function mutations of *fem-3*, a sex-determination gene in *Caenorhabditis elegans*. Genetics **115:** 107–119.

Blelloch, R., and J. Kimble, 1999 Control of organ shape by a secreted metalloprotease in the nematode *Caenorhabditis elegans*. Nature **399:** 586–590.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

C. elegans Sequencing Consortium, 1998 Genome sequence of the nematode *C. elegans*: a platform for investigating biology. Science **282:** 2012–2018.

Chamberlin, H. M., R. E. Palmer, A. P. Newman, P. W. Sternberg, D. L. Baillie *et al.*, 1997 The PAX gene *egl-38* mediates developmental patterning in *Caenorhabditis elegans*. Development **124:** 3919–3928.

Gu, G., G. A. Caldwell and M. Chalfie, 1996 Genetic interactions affecting touch sensitivity in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **93:** 6577–6582.

Hodgkin, J. A., H. R. Horvitz and S. Brenner, 1979 Nondisjunction mutants of the nematode *Caenorhabditis elegans*. Genetics **91:** 67–94.

Hosono, R., K. Hirahara, S. Kuno and T. Kurihara, 1982 Mutants of *Caenorhabditis elegans* with dumpy and rounded head phenotype. J. Exp. Zool. **224:** 135–144.

Kelly, W. G., and A. Fire, 1998 Chromatin silencing and the maintenance of a functional germline in *Caenorhabditis elegans*. Development **125:** 2451–2456.

Kelly, W. G., S. Xu, M. K. Montgomery and A. Fire, 1997 Distinct requirements for somatic and germline expression of a generally expressed *Caenorhabditis elegans* gene. Genetics **146:** 227–238.

Korswagen, H. C., R. M. Durbin, M. T. Smits and R. H. A. Plasterk, 1996 Transposon Tc1-derived, sequence-tagged sites in *Caenorhabditis elegans* as markers for gene mapping. Proc. Natl. Acad. Sci. USA **93:** 14680–14685.

Kramer, J. M., R. P. French, E. C. Park and J. J. Johnson, 1990 The *Caenorhabditis elegans rol-6* gene, which interacts with the *sqt-1* collagen gene to determine organismal morphology, encodes a collagen. Mol. Cell. Biol. **10:** 2081–2090.

Kyriakis, J. M., and J. Avruch, 1996 Protein kinase cascades activated by stress and inflammatory cytokines. Bioessays **18:** 567–577.

LaMunyon, C. W., and S. Ward, 1994 Assessing the viability of mutant and manipulated sperm by artificial insemination of *Caenorhabditis elegans*. Genetics **138:** 689–692.

L'Hernault, S. W., D. C. Shakes and S. Ward, 1988 Developmental genetics of chromosome I spermatogenesis-defective mutants in the nematode *Caenorhabditis elegans*. Genetics **120:** 435–452.

Leberer, E., D. Y. Thomas and M. Whiteway, 1997 Pheromone signalling and polarized morphogenesis in yeast. Curr. Opin. Genet. Dev. **7:** 59–66.

Ma, C., H. Liu, Y. Zhou and K. Moses, 1996 Identification and characterization of autosomal genes that interact with *glass* in the developing Drosophila eye. Genetics **142:** 1199–1213.

Machaca, K., L. J. DeFelice and S. W. L'Hernault, 1996 A novel chloride channel localizes to *Caenorhabditis elegans* spermatids and chloride channel blockers induce spermatid differentiation. Dev. Biol. **176:** 1–16.

Madden, K., and M. Snyder, 1998 Cell polarity and morphogenesis in budding yeast. Annu. Rev. Microbiol. **52:** 687–744.

Maloof, J. N., J. Whangbo, J. M. Harris, G. D. Jongeward and C. Kenyon, 1999 A Wnt signaling pathway controls HOX gene expression and neuroblast migration in *C. elegans*. Development **126:** 37–49.

Mello, C., and A. Fire, 1995 DNA transformation, pp. 452–482 in *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, edited by H. F. Epstein and D. C. Shakes. Academic Press, San Diego.

Minniti, A. N., C. Sadler and S. Ward, 1996 Genetic and molecular analysis of *spe-27*, a gene required for spermatogenesis in *Caenorhabditis elegans* hermaphrodites. Genetics **143:** 213–223.

Nance, J., A. N. Minniti, C. Sadler and S. Ward, 1999 *spe-12* encodes a sperm cell surface protein that promotes spermiogenesis in *Caenorhabditis elegans*. Genetics **152:** 209–220.

Nelson, G. A., and S. Ward, 1980 Vesicle fusion, pseudopod extension and amoeboid motility are induced in nematode spermatids by the ionophore monensin. Cell **19:** 457–464.

Nelson, G. A., K. K. Lew and S. Ward, 1978 *Intersex*, a temperature-sensitive mutant of the nematode *C. elegans*. Dev. Biol. **66:** 386–409.

Page, B. D., W. Zhang, K. Steward, T. Blumenthal and J. R. Priess, 1997 ELT-1, a GATA-like transcription factor, is required for epidermal cell fates in *Caenorhabditis elegans* embryos. Genes Dev. **11:** 1651–1661.

Pavalko, F. M., and T. M. Roberts, 1989 Posttranslational insertion of a membrane protein on *Caenorhabditis elegans* sperm occurs without *de novo* protein synthesis. J. Cell. Biochem. **41:** 57–70.

Qu, C. K., W. M. Yu, B. Azzarelli and G. S. Feng, 1999 Genetic evidence that Shp-2 tyrosine phosphatase is a signal enhancer of the epidermal growth factor receptor in mammals. Proc. Natl. Acad. Sci. USA **96:** 8528–8533.

Shakes, D. C., and S. Ward, 1989 Initiation of spermiogenesis in *C. elegans*: a pharmacological and genetic analysis. Dev. Biol. **134:** 189–200.

Thomas, J. H., 1993 Thinking about genetic redundancy. Trends Genet. **9:** 395–399.

Ward, S., 1986 Asymmetric localization of gene products during the development of *Caenorhabditis elegans* spermatozoa, pp. 55–75 in *Gametogenesis and the Early Embryo*. Alan R. Liss, New York.

Ward, S., and J. S. Carrel, 1979 Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. Dev. Biol. **73:** 304–321.

Ward, S., and J. Miwa, 1978 Characterization of temperature-sensitive, fertilization-defective mutants of the nematode *Caenorhabditis elegans*. Genetics **88:** 285–303.

Ward, S., Y. Argon and G. A. Nelson, 1981 Sperm morphogenesis in wild-type and fertilization-defective mutants of *Caenorhabditis elegans*. J. Cell Biol. **91:** 26–44.

Ward, S., E. Hogan and G. A. Nelson, 1983 The initiation of spermiogenesis in *Caenorhabditis elegans*. Dev. Biol. **98:** 70–79.

Williams, B. D., 1995 Genetic mapping with polymorphic sequence-tagged sites, pp. 81–96 in *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, edited by H. F. Epstein and D. C. Shakes. Academic Press, San Diego.

Williams, B., B. Schrank, C. Huynh, R. Shownkeen and R. H. Waterston, 1992 A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. Genetics **131:** 609–624.

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