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A sperm-supplied factor required for embryogenesis in *C. elegans*

Heidi Browning* and Susan Strome

Department of Biology and Institute for Molecular and Cellular Biology, Indiana University, Bloomington, IN 47405, USA

*Present address: Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309, USA

SUMMARY

The paternal-effect embryonic-lethal gene, *spe-11*, is required for normal development of early *C. elegans* embryos. Spe-11 embryos fail to complete meiosis, form a weak eggshell, fail to orient properly the first mitotic spindle, and fail to undergo cytokinesis. Here we report cloning and sequencing of the *spe-11* gene, which encodes a novel protein. As predicted by the paternal-effect mutant phenotype, the gene is expressed during spermatogenesis but is not detectable in females undergoing oogenesis, and the protein is present in mature sperm. To investigate whether SPE-11's essential function is during spermatogenesis or whether sperm-delivered SPE-11 functions in the newly fertilized embryo, we engineered animals to supply SPE-11 to the embryo through the oocyte rather than through the sperm. We found that maternal expression is sufficient for embryonic viability. This result demonstrates that SPE-11 is not required during spermatogenesis, and suggests that SPE-11 is a sperm-supplied factor that participates directly in development of the early embryo. In contrast to the many known maternal factors required for embryogenesis, SPE-11 is the first paternally contributed factor to be genetically identified and molecularly characterized.

Key words: *C. elegans*, *spe-11*, sperm-supplied factor, embryogenesis

INTRODUCTION

At fertilization the sperm and oocyte fuse to form the zygote, each gamete providing components necessary for development of the embryo. In many organisms large stockpiles of RNAs and proteins are synthesized by the oocyte and/or supporting cells before fertilization (Davidson, 1986). In addition, analyses of maternal-effect embryonic-lethal mutants in *C. elegans* and *Drosophila* have revealed that many embryonic events are guided by maternally provided factors (reviewed by St Johnston and Nusslein-Volhard, 1992; Wood and Edgar, 1994).

Although fewer in number, sperm contributions are essential for normal embryogenesis in most organisms: (1) The sperm supplies the signal for initiation of the embryonic program. The exact mechanism of egg activation is not well understood, but in many organisms it results in a block to polyspermy, a transient rise in intracellular [Ca^{2+}], a permanent rise in pH, completion of the maternal meiotic cell cycle, an increase in protein synthesis, and entrance into the mitotic cell cycle (reviewed by Davidson, 1986; Kline, 1991; Nuccitelli, 1991; Whitaker and Swann, 1993). (2) In general, centrosomes are paternally inherited (Schatten, 1994). Centrosomes are responsible for nucleating the microtubule array used for pronuclear migration as well as the microtubules in the mitotic spindle. In addition, in *Xenopus* the sperm aster is thought to be involved in the cortical rotation that specifies the dorsal side of the embryo (reviewed by Houliston and Elinson, 1992). (3) The sperm and oocyte each provide a haploid genome in order to produce a diploid zygote. In many organisms, as a result of differential genomic imprinting, the maternally and paternally derived genomes are not equivalent (reviewed by Monk and Surani, 1990).

Observations of early embryonic events by microscopy suggest that the sperm of *Caenorhabditis elegans* provides all three of the previously discussed sperm-supplied components and signals. The sperm provides the signal for embryogenesis to begin. Shortly after sperm entry an eggshell begins to form and the oocyte nucleus, which had arrested in diakinesis of prophase I, completes the first and second meiotic divisions (Hirsh et al., 1976). In the absence of sperm, oocytes complete only the first meiotic division and do not form an eggshell (Ward and Carrel, 1979). Unfertilized oocytes undergo rounds of DNA replication, but in the absence of the sperm-supplied centrosome do not undergo mitosis (Ward and Carrel, 1979; Albertson, 1984). In fertilized embryos the sperm-supplied centrosome duplicates, and microtubules that nucleate from the resulting centrosomes participate in pronuclear migration and formation of the first mitotic spindle (Albertson, 1984; Strome and Wood, 1983). The sperm also contributes a haploid genome. Genetic analysis indicates that the maternally and paternally contributed genomes of *C. elegans* are equivalent, and thus do not appear to be subject to differential parental imprinting (Haack and Hodgkin, 1991).

Most of the current information about paternal contributions to embryogenesis has come from molecular, biochemical and cell biological studies of fertilization and embryogenesis. Genetic identification of paternal-effect embryonic-lethal (PEL) mutants and molecular and cellular analysis of the genes identified offer a complementary approach. Three PEL genes

have been genetically identified: *pal* (Baker, 1975) and *ms(3)K81* (Fuyama, 1986; Yasuda et al., 1995) in *Drosophila* and *spe-11* (Hill et al., 1989) in *C. elegans*. Phenotypic analyses of the *Drosophila* PEL genes suggest that *pal* is involved in male chromosome maintenance (Baker, 1975) and *ms(3)K81* is involved in controlling the behavior of the paternal pronucleus (Fuyama, 1986; Yasuda et al., 1995). The *spe-11* gene product appears to be directly or indirectly involved in a diverse array of events in the one-cell embryo (Hill et al., 1989).

The *spe-11* gene of *C. elegans* was identified in a genetic screen for spermatogenesis-defective mutants (L'Hernault et al., 1988). Mutations in *spe-11* result in a strict paternal-effect embryonic-lethal phenotype: fertilization of wild-type oocytes by sperm from homozygous mutant animals leads to abnormal early embryonic development, while fertilization of mutant oocytes by wild-type sperm leads to normal development (Hill et al., 1989). All the sperm from heterozygous males are able to father viable progeny when mated to homozygous mutant hermaphrodites. The results of these genetic crosses illustrate that: (1) *spe-11* mutations are recessive and therefore the mutant genes are not likely to encode poisonous products, (2) paternal expression of the *spe-11* gene is both necessary and sufficient for normal embryogenesis to occur, (3) the Spe-11 phenotype is not the result of defective genomic imprinting of the *spe-11* locus, since progeny that receive a mutant allele from heterozygous fathers develop normally.

Spe-11 embryos (i.e. embryos fertilized by sperm from homozygous mutant animals) display early and severe defects (Hill et al., 1989). They fail to complete meiosis, form a weak eggshell, undergo an abnormal pseudocleavage, fail to orient properly the first mitotic spindle, and fail to undergo cytokinesis. They continue to undergo several rounds of mitosis without cytokinesis, developing into multinucleate, single-cell embryos.

The only defect seen in sperm from *spe-11* animals is in the electron-dense halo that surrounds the nucleus and centriolar pair (Hill et al., 1989). In wild-type sperm this halo is evenly distributed around the nucleus and centriolar pair (Wolf et al., 1978), while in sperm from animals bearing the three severe alleles of *spe-11* this material is unevenly distributed and contains extra granular material (Hill et al., 1989 and H.B., C. Sganga, F. R. Turner and S.S., unpublished result). The halo appears normal in sperm from animals bearing the *hc77*ts allele of *spe-11*, suggesting that the aberrant halo is not responsible for the embryonic-lethal phenotype (Hill et al., 1989).

In this paper, we have analyzed *spe-11* at the molecular and cellular level. As predicted by the paternal-effect mutant phenotype, the gene is expressed during spermatogenesis but not detectable in females undergoing oogenesis. Consistent with the aberrant halo phenotype, SPE-11 is localized to the perinuclear region of sperm. To investigate whether SPE-11's essential function is during spermatogenesis, to make sperm capable of properly participating in embryogenesis, or whether sperm-delivered SPE-11 functions directly in the newly fertilized embryo, we expressed the *spe-11* gene in oocytes and fertilized those oocytes with sperm from *spe-11* mutant animals. Many of the fertilized embryos developed normally. This suggests that SPE-11 is not required during spermatogenesis, and that it participates directly in the early events of embryogenesis.

MATERIALS AND METHODS

Maintenance of strains

All strains used were generated from the N2 Bristol variety. Strains were maintained as described by Brenner (1974). The following mutations were used in this study. LGI: *spe-11(hc90), dpy-5(e61), unc-73(rh40), glp-4(bn2*ts*).* LGIII: *fem-2(b245*ts)lf. LGIV: *him-8(e1489), fem-3(q20*ts)gf. The LGI duplication *hDp29* was also used. Some strains were provided by the *Caenorhabditis* Genetics Center (CGC).

Construction and screening of a lambda phage library

An unamplified lambda phage library was constructed using intact high molecular mass genomic DNA. DNA was prepared by proteinase K digestion of worms embedded in agarose (to be described in detail elsewhere; H. B., C. Madej, L. Berkowitz, J. Paulsen, M. Zolan, and S. S., unpublished data). The embedded DNA was partially digested with *Sau*3AI restriction enzyme and size-fractionated on a low melting point agarose gel. DNA in the size range 15-23 kb was isolated from the gel by agarase treatment followed by a series of phenol, phenol/chloroform, and chloroform extractions. The isolated DNA was dephosphorylated and ligated into the *Bam*HI cloning site of the lambda DASH II cloning vector (Stratagene). Phage were packaged using Stratagene gigapack II XL packaging extract. The *E. coli* strain SRP(P2) (Stratagene) was infected with the phage. The library was screened using standard molecular techniques (Sambrook et al., 1989). YACs and cosmids were radiolabeled with [α-^{32}P]dCTP using the Boehringer Mannheim Random Primed Labeling Kit. The rescuing phage was subcloned into pBluescript KS$^+$ and SK$^+$ (Stratagene).

Preparation of DNA for microinjection

To obtain YAC DNA, yeast chromosomal DNA was prepared as described by Schwartz and Cantor (1984) with modifications described in the Biorad CHEF DRII instruction manual. The intact chromosomal DNA was size-fractionated on an agarose gel using a Biorad CHEF DRII apparatus. The YAC was excised from the gel and purified using the BIO101 gene clean kit.

For preparation of phage DNA, SRP(P2) *E. coli* were infected as described by Malik et al. (1990). Phage, cosmid, and plasmid DNA were isolated from *E. coli* as described by Sambrook et al. (1989).

Transformation rescue

Transgenic lines were established by the method of Mello et al. (1991). *unc-73(rh40)++/+spe-11(hc90)dpy-5(e61)* hermaphrodites were injected with test DNA (1-250 ng/µl) and a marker for transformation, pRF4 (approximately 100 ng/µl). Heritable lines containing extrachromosomal arrays of injected DNA were established, and *spe-11dpy-5* hermaphrodites were tested for the ability to produce viable progeny. The Rol phenotype conferred by pRF4 is suppressed by the Dpy-5 phenotype, and as a result both transformed and untransformed hermaphrodites were tested for rescue. Although many *spe-11dpy-5* hermaphrodites were tested for each line, the number of lines that rescue is a minimum because it is possible that transformed hermaphrodites were not tested for every line. Recombination between *spe-11* and *dpy-5* was monitored by checking for Dpy Unc progeny in potentially rescued broods.

Rescue with the YAC Y51G8 was complicated because it also rescues Dpy-5. The first indication that this was occurring was that transformed *unc-73++/+spe-11dpy-5* hermaphrodites produced less than 1/4 Dpy progeny. Furthermore, some phenotypically wild-type animals from such a brood produced no Unc and few or no Dpy progeny. To demonstrate that these animals were homozygous *spe-11dpy-5* and rescued for both Spe-11 and Dpy-5, the putative rescued hermaphrodites were crossed to wild-type males. F_1 hermaphrodites were placed on individual plates, and the phenotype of their progeny

was determined. With the exception of a few animals that produced too few progeny to assess, all F_1 hermaphrodites produced some Dpy-5 Spe-11 progeny.

cDNA isolation and sequencing

The 2.8 kb *Eco*RI-*Sal*I rescuing genomic fragment was used to screen a male-enriched cDNA library (kindly provided by J. Varkey and S. Ward). Seventeen cDNAs were isolated, and the 5′ ends of the 8 largest were sequenced to determine which cDNA is the longest. Both strands of the longest cDNA and the corresponding genomic region were sequenced in their entirety. The cDNA contained a polyA tract at the 3′ end. DNA was sequenced using the USB Sequenase Version 2.0 Sequencing kit. The *hc90* allele of *spe-11* was sequenced as described by Perry et al. (1994).

Searches of sequence databases were performed using the FASTA (Pearson and Lipman, 1988), BLAST (Altschul et al., 1990), BLITZ (Sturrock and Collins, 1993), and MacPattern (Bairoch, 1990; Fuch, 1991) programs.

Northern analysis

RNA was isolated from populations of synchronously growing worms essentially as described by Rosenquist and Kimble (1988). Synchronization was achieved by treating adult hermaphrodites with hypochlorite (Johnson and Hirsh, 1979), allowing the resulting embryos to hatch in the absence of food, and transferring the arrested L1s to food. Embryos were staged by DAPI staining or Nomarski microscopy of a small sample of the embryos collected for RNA isolation. poly(A)$^+$ RNA was isolated using the Promega PolyATtract mRNA Isolation System III kit. Approximately 5 μg of poly(A)$^+$ RNA were electrophoresed on a 1.2% agarose 6% formaldehyde gel. Hybridization and washes were a slight modification of the procedure described by Peden et al. (1982). Northern blots were prehybridized in 1× Denhardts, 1 M NaCl, 50 mM $NaPO_4$, pH 6.5, 5 mM EDTA, pH 8.0, 10% dextran sulfate, and 20 μg/ml denatured salmon sperm DNA at 60°C for several hours. The *spe-11* cDNA was radiolabeled with [α-^{32}P]dCTP using the Boehringer Mannheim Random Primed Labeling Kit. Denatured probe was added to the prehybridization solution, and hybridization was allowed to proceed overnight. The blots were washed at 68°C for 1 hour in 1 M NaCl, 50 mM Tris, pH 8.5, 2 mM EDTA, pH 8.0, 1% SDS, followed by 1 hour in 0.5 M NaCl, 50 mM $NaPO_4$, pH 6.5, and 0.5% SDS. Some blots were washed further in 0.5× SSC or 0.2× SSC, 0.1% SDS.

Generation and purification of antisera

Two SPE-11 fusion protein constructs were made, pGEXKG::*spe-11* and pMALc::*spe-11*. For the construction of pGEXKG::*spe-11*, the *spe-11* cDNA coding region was excised from pBluescript SK$^-$ by digestion with *Eco*RI and *Xho*I and cloned into the *Eco*RI and *Xho*I sites of pGEXKG (Guan and Dixon, 1991). For the construction of pMALc::*spe-11*, the *spe-11* cDNA coding region was excised from pBluescript SK$^-$ by digestion with *Eco*RI and *Xho*I, 5′ overhangs were filled in using the Klenow fragment of DNA polymerase I, and the resulting fragment was ligated into the *Stu*I site of pMALc (New England Biolabs).

GST::SPE-11 fusion protein was produced in *E. coli* and purified using glutathione agarose beads (Ausubel et al., 1988; Guan and Dixon, 1991). After washing, the beads were resuspended in loading buffer, placed in a boiling water bath for 3 minutes to dissociate the fusion protein from the beads, and size-fractionated by SDS polyacrylamide gel electrophoresis. Gel slices containing fusion protein and electroeluted soluble protein were sent to the Pocono Rabbit Farm for immunization of three rats.

Sera from all three rats were affinity purified against MBP::SPE-11 fusion protein, produced in *E. coli* from pMALc::*spe-11* (Ausubel et al., 1988). Bacterial pellets of *E. coli* expressing MBP::SPE-11 were resuspended in loading buffer, placed in a boiling water bath for 3 minutes, and electrophoresed on an SDS polyacrylamide gel. The size-fractionated extract was electrophoretically transferred to a nitrocellulose membrane (Towbin et al., 1979). The nitrocellulose membrane was stained with Ponceau S, and a band present in induced but not in uninduced extracts and of the appropriate size for the fusion protein was excised. Sera were blot affinity purified as described by Olmsted (1986).

Western blot analysis of affinity purified sera

To prepare protein homogenates, synchronized populations of worms (synchronized by hypochlorite treatment as described above) were collected by centrifugation. The worm pellet was added to an equal volume of homogenization buffer (3% SDS, 63 mM Tris pH 6.8, 0.72 M 2-mercaptoethanol, 16% glycerol, 1 mg/ml bromophenol blue, 2 mM PMSF, 20 μg/ml pepstatin, 20 μg/ml leupeptin, 0.034 TIU/ml aprotinin) in a dounce homogenizer in a boiling water bath. The suspension was heated for 1 minute in the boiling water bath, homogenized, then heated for an additional 3 minutes. The mixture was subjected to low speed centrifugation, and the supernatant was stored in aliquots at −70°C.

Western blot analysis was performed as described by Towbin et al. (1979), except that the pH of the transfer buffer was adjusted to 10.5 with NaOH to facilitate transfer of SPE-11. (The isoelectric point of SPE-11 is pH 7.93 as calculated by the Genetics Computer Group ISOELECTRIC program.) After transfer, blots were stained with Ponceau S, destained in double distilled H_2O, then washed in several changes of 1× PBS (phosphate-buffered saline). Blots were blocked for at least 1 hour at 37°C in 1× PBS with 3% BSA and 5% goat serum; reacted with a 1:50 dilution of purified sera in 1× PBS, 0.1% Triton X-100 with 1.5% BSA and 5% goat serum overnight at 4°C; washed in 1× PBS, 0.1% Triton X-100 at room temperature; reacted with a 1:5000 dilution of alkaline phosphatase-conjugated affinipure goat anti-rat antibody (Jackson Labs) in 1× PBS, 0.1% Triton X-100 with 1.5% BSA and 5% goat serum at room temperature for at least 1 hour; washed in 1× PBS, 0.1% Triton X-100 at room temperature. Bound antibodies were visualized as described by Harlow and Lane (1988).

Immunofluorescence microscopy

Males and hermaphrodites were fixed and stained as described by Strome and Wood (1983). The DNA in samples for confocal microscopy was stained with propidium iodide as follows: either before reaction with primary antibody or after washing off unbound secondary antibody, samples were RNAse treated as described by Edgar and McGhee (1988) by incubating at 37°C for 1 hour in 2× SSC, 0.1 mg/ml RNAse A. After RNAse treatment, samples were washed several times in 1× PBS. Samples were mounted in 4 μg/ml propidium iodide in gelutol. Nuclear pore complex antibody mAb414 (Berkeley Antibody Company) was used at a 1:200 dilution. Secondary antibodies (Jackson Labs) were used at a 1:100-1:200 dilution. Samples were examined by epifluorescence microscopy using a Zeiss Axioskop or by confocal microscopy using a Biorad 600 Confocal Microscope.

Photography and confocal image processing

Conventional immunofluorescence micrographs were taken with Kodak Tri-X 100 pan film (ASA1600) and developed using Diafine two bath developer (Acufine, Inc.). Confocal images were collected using an IBM computer, transferred to a Power Macintosh 7100/66, and opened using the NIH Image program (written by Wayne Rasband at the U.S. National Institutes of Health and available from the Internet by anonymous ftp from zippy.nimh.nih.gov or on floppy disk from NTIS, 5285 Port Royal Rd., Springfield, VA 22161, part number PB93-504868). Images were imported into Adobe Photoshop. The brightness and contrast of the images were adjusted using Adobe Photoshop. The images were also merged using Adobe Photoshop.

Ectopic expression experiments

The hsp16 promoter cassette vector, pPD49.78 (gift from A. Fire), contains the *C. elegans* hsp16 promoter, a 42 bp synthetic intron, and 3′ polyadenylation signal sequences from *unc-54* (in the order described). The *spe-11* cDNA was inserted between the synthetic intron and the *unc-54* 3′ sequences. The construction was as follows: the *spe-11* cDNA was excised from pBluescript SK⁻ by restriction digestion with *Eco*RI and *Xho*I and the 5′ overhangs were filled in using Klenow, pPD49.78 was restriction digested with *Kpn*I and the 3′ protruding overhang was digested using Klenow, and the blunt-ended *spe-11* cDNA was ligated into this blunted site.

N2 hermaphrodites were transformed with phsp16::*spe-11*cDNA along with a marker for transformation, pRF4 (Mello et al., 1991). Heritable lines were established. Gravid, transformed hermaphrodites were heat shocked for 2 hours at 33-35°C. After heat shock, the animals were allowed to recover for at least 10 minutes, then fixed and stained with anti-SPE-11 antibody (Strome and Wood, 1983).

Oocyte rescue experiment

The syncytial ovaries of adult *spe-11(hc90)dpy-5(e61)* hermaphrodites were injected with the phsp16::*spe-11*cDNA (described above) or control DNA (a pBluescript clone of a genomic region downstream of *spe-11*) at a concentration of approximately 25 ng/μl. The animals were allowed to recover from the injection for 1.5-2 hours at 16°C. (Animals that appeared to have moved very little during this recovery time were judged not to have adequately recovered from the injection procedure and were discarded.) After the recovery period, animals were either heat shocked for 2 hours at 33-35°C or left at room temperature. Several days later the number of viable progeny produced by each injected worm was counted. In experiments in which injected animals were mated to *spe-11* males, the males were placed on the plates after the heat shock and removed approximately 20 hours later. *spe-11(hc90)+/spe-11(hc90)dpy-5(e61)* males were generated as follows: *spe-11(hc90)dpy-5(e61)/spe-11(hc90)dpy-5(e61)* hermaphrodites were crossed to *unc-73(rh40)+dpy-5(e61)/+spe-11(hc90)+ ; him-8(e1489)* males. Male progeny that were not Dpy were used for crosses.

Heritable lines carrying phsp16*spe-11*cDNA and pRF4 were established as described for transformation rescue. *spe-11(hc90)dpy-5(e61)* hermaphrodites were heat shocked as young adults for 2 hours at 32-35°C. A *spe-11dpy-5* transformed line capable of producing viable progeny after heat shock was established. Crosses to *spe-11* males were performed as described above.

RESULTS

Spermatogenesis in *C. elegans* (background)

Spermatogenesis in *C. elegans* has been described in detail (Klass et al., 1976; Wolf et al., 1978; Ward et al., 1981; Kimble and Ward, 1988). We briefly review here the events relevant to this paper. There are two naturally occurring sexes in *C. elegans*, the hermaphrodite and the male. Hermaphrodites begin spermatogenesis during the fourth larval stage and as young adults convert to oogenesis. Males begin spermatogenesis during the fourth larval stage and continue to produce sperm throughout their adult life. Spermatogenesis proceeds sequentially along the single gonad arm of the male and both gonad arms of the hermaphrodite (Fig. 1A,B). In the most distal region of the gonad, the germ cells undergo mitotic divisions. Meiotic pachytene nuclei are in and beyond the loop region. Both meiotic divisions of spermatogenesis occur in a narrow region in the proximal arm of the gonad. After the first meiotic division, cytokinesis is sometimes incomplete, leaving the two secondary spermatocytes attached. Following the second meiotic division, a cytoplasmic structure, termed the residual body, forms between two (complete cytokinesis) or four (incomplete cytokinesis) haploid spermatids. Many cellular components are segregated to the residual body, including the ribosomes, microfilaments, microtubules, Golgi apparatus, and most of the intracellular membranes. After budding from the residual body, the spermatid undergoes spermiogenesis, forming a spermatozoan with a pseudopod used for motility. Spermatids and spermatozoa are thought to be transcriptionally and translationally quiescent. Interestingly, no nuclear membrane is visible by electron microscopy (Wolf et al., 1978; Kimble and Ward, 1988) or antibody staining (Fig. 6D) in spermatids or spermatozoa. (In this paper we will use the term ‘sperm’ in situations in which we have not been able to discriminate between spermatids and spermatozoa.)

Molecular identification of the *spe-11* gene

The *spe-11* gene was molecularly identified by transformation rescue (Fig. 2). Most of the *C. elegans* genome has been cloned into yeast artificial chromosomes (YACs), cosmids, and phage, which have been assembled into contiguous regions of overlapping clones designated ‘contigs’ (Coulson et al., 1986, 1988, 1991). *spe-11* was previously genetically mapped between *unc-38* and *dpy-5* (L’Hernault et al., 1988). Both *unc-38* and *dpy-5* have been placed on the physical map on a single contig (J. Lewis, personal communication; Babity, 1993). Clones between these two genes were used to generate transgenic lines by the method of Mello et al. (1991), and transgenic *spe-11(hc90)* hermaphrodites were tested for their ability to produce viable progeny. The 230 kb YAC Y51G8 rescued the mutant phenotype. However, none of the cosmids shown in Fig. 2 rescued the mutant phenotype. Because we found that some clones in this region are prone to rearrangement, we chose to use lambda phage clones instead of additional cosmid clones to further characterize the region containing the *spe-11* gene. An unamplified lambda phage library was made from genomic DNA and screened with the rescuing YAC Y51G8 and the YAC Y38H4, which ends in the cosmid that contains *dpy-5* (Babity, 1993; H.B., unpublished result). Phage that hybridized to Y51G8 but not to Y38H4 were positioned on the physical map by hybridizing cosmids from this region to a Southern blot of restriction digested phage. The phage shown in Fig. 2 were tested by transformation rescue. Phage 21 rescued the Spe-11 phenotype. The genomic region containing the *spe-11* gene was further defined by testing restriction fragments and/or subclones of this phage for transformation rescue. The smallest rescuing, genomic region identified is a 2.3 kb *Cla*I-*Sal*I subclone.

The 2.8 kb *Eco*RI-*Sal*I rescuing subclone was used to screen a male-enriched cDNA library (kindly provided by Jacob Varkey and Samuel Ward). The evidence that the cDNA identified by the rescuing fragment codes for SPE-11 is that expression of the cDNA in transgenic animals can rescue the mutant phenotype (see later section), and sequencing of the corresponding region from DNA from *spe-11(hc90)* animals identified two base pair changes. The change of a G to an A at nucleotide 1322 (see sequence in Fig. 3) replaces the tryptophan at position 191 with a stop codon. The other mutation results in a conservative amino acid transition.

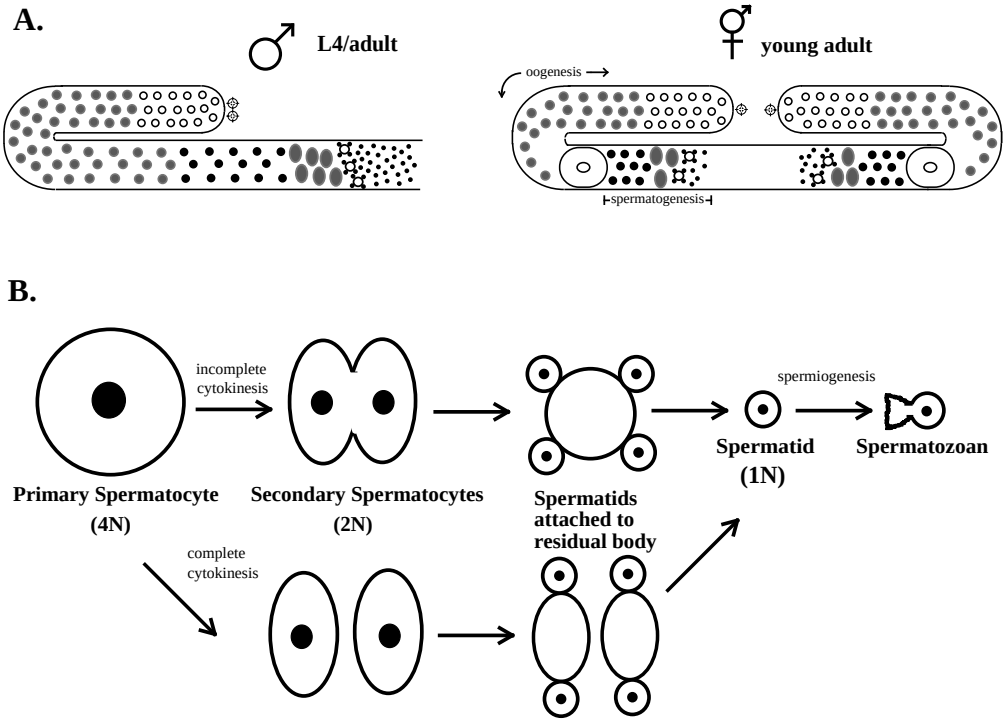

Fig. 1. Schematic representation of spermatogenesis in L4 and adult males and young adult hermaphrodites. (A) Gonad arms of the male and hermaphrodite. Spermatogenesis proceeds temporally along the single gonad arm of the male and both gonad arms of the hermaphrodite. For a short period of time the young adult hermaphrodite is engaged in both oogenesis and spermatogenesis as diagrammed here. (⊕) Somatic gonad distal tip cell, (○) mitotic nuclei, (●) early meiotic nuclei (mostly pachytene), (●) primary spermatocytes, (●) secondary spermatocytes, (⌘) spermatids attached to residual body, (•) sperm, (⊙) oocytes. (Adapted from Kimble and White, 1981.) (B) Stages of spermatogenesis. After the first meiotic division cytokinesis is often incomplete. Following the second meiotic division, many cytoplasmic components remain in the residual body. The spermatids bud from the residual body and form a pseudopod used for motility. (Adapted from L'Hernault et al., 1988.)

spe-11 encodes a novel protein

The cDNA and corresponding genomic sequence are shown in Fig. 3A. The longest open reading frame codes for a 299 amino acid protein (Fig. 3A). Searches of protein databases do not reveal significant similarity to previously identified proteins. As demonstrated by the Kyte-Doolittle hydropathy plot (Fig. 3B), the protein is very hydrophilic. This is due primarily to the numerous acidic and basic residues: 41% of the protein is composed of arginine, lysine, glutamic acid and aspartic acid residues.

The *spe-11* gene is expressed during spermatogenesis

Northern hybridization analysis was performed to determine the expression pattern of the *spe-11* gene. This analysis revealed that in hermaphrodites the transcript is most abundant during the fourth larval stage and is present at lower levels in adults (Fig. 4A). Transcription during spermatogenesis (the fourth larval stage) is consistent with the paternal-effect phenotype (Hill et al., 1989). The presence of the transcript in adults could be due to one or more of the following: (1) transcription in the maternal germline, (2) presence of the transcript in tissues other than the germline, (3) transcription in young adults completing spermatogenesis, (4) presence of the transcript in spermatozoa. To eliminate some of these possibilities, further analysis was performed.

To determine if the transcript is present in the maternal germline or other tissues of the adult, northern analysis was performed on RNA from *fem-2(b245*ts)lf adults, which produce oocytes but no sperm (Kimble et al., 1984), and *glp-4(bn2*ts) adults, which have a severely reduced and undifferentiated germline (Beanan and Strome, 1992). The *spe-11* transcript was not detectable in adults from either of these strains (Fig. 4B). Thus, the presence of the transcript in adults is dependent on the presence of sperm. This correlation was further demonstrated by analysis of RNA from *fem-3(q20*ts)gf adults, which overproduce sperm and never switch to oogenesis (Barton et al., 1987). *fem-3*gf adults contain a high level of *spe-11* transcript compared to wild-type adults (Fig. 4B).

To address whether the transcript present in adults is due solely to completion of spermatogenesis in young adults or also to the presence of the transcript in mature sperm, young embryos were examined for the presence of the transcript. If *spe-11* RNA is present in mature sperm, it may be delivered to the embryo by the sperm and may be detectable in RNA from early embryos. Northern analysis of RNA from different stage embryos demonstrates that the *spe-11* transcript is indeed detectable in very young embryos but not in older embryos (Fig. 4C). It is possible that this embryonic transcript is the result of embryonic transcription. However, because of the high level of *spe-11* transcription during sper-

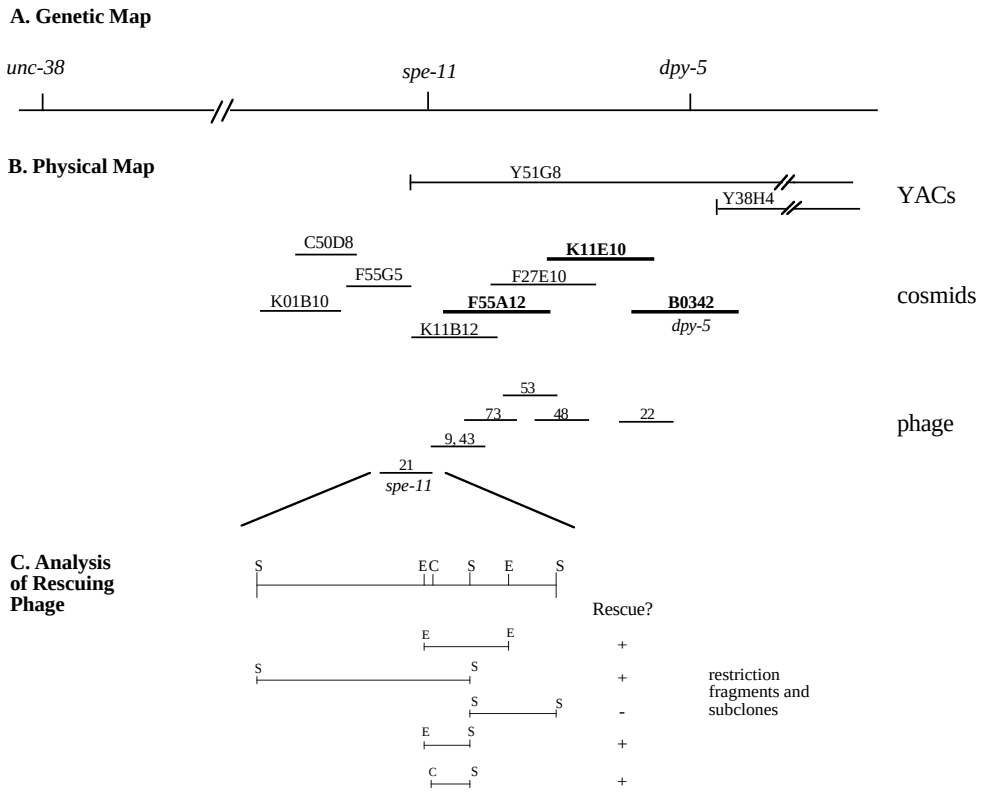

Fig. 2. Genetic and physical map of the *spe-11* region. (A) Genetic map. *spe-11* maps 0.1-0.2 map units left of *dpy-5*, between *dpy-5* and *unc-38*. (B) Physical map. The cosmid and YAC clones shown were used for transformation rescue and lambda phage library screening. Y51G8 rescued the Spe-11 phenotype. The cosmid clones shown did not rescue the mutant phenotype. Lambda phage that hybridized to Y51G8 but not Y38H4 were isolated from an unamplified lambda phage library. The phage were positioned on the contig by hybridization to the cosmids shown in bold and tested for transformation rescue. Phage 21 rescued the mutant phenotype. Although K11B12 and F55G5 hybridize to part of phage 21, neither rescued the Spe-11 phenotype. They may not have rescued because they do not contain part or all of the gene, perhaps because of a deletion in our isolates of the cosmids. (C) Analysis of rescuing phage. Subclones and restriction fragments of phage 21 were tested for transformation rescue. The numbers of rescued lines/number of lines tested for the subclones were as follows: 8/8 for the E-E subclone, 17/18 for the E-S subclone, and 3/4 for the C-S subclone. These represent minimum values (see Materials and Methods for details). The number of viable progeny produced by rescued animals varied greatly from animal to animal within the same heritable line, possibly due to the stability of the array in the germline. However, some animals transformed with the 2.3 kb C-S subclone were capable of producing nearly the same number of viable progeny as *spe-11+* animals in the same genetic background. Flanking *Sal*I sites are from the phage polylinker. Restriction sites: S, *Sal*I; E, *Eco*RI; C, *Cla*I. (Additional E and C sites not shown.)

matogenesis and the presence of the transcript only in young embryos, we think the most likely source is the sperm. Thus, the presence of the *spe-11* RNA in adults is likely due to both the completion of spermatogenesis and the presence of the transcript in mature sperm. Taken together, these results demonstrate that the *spe-11* gene is transcribed during spermatogenesis and that transcription of the gene is probably restricted to this process.

Western blot analysis of antisera to SPE-11

To analyze the *spe-11* gene product at the protein level, antibodies were raised to a glutathione S-transferase-SPE-11 fusion protein. Sera from three immunized rats were blot affinity purified against a maltose-binding protein-SPE-11 fusion protein. All three purified sera react with a single 36×10^3 M_r protein on western blots of homogenate from wild-type hermaphrodites (data not shown). Western analysis using purified serum illustrates that the presence of this protein correlates with the presence of sperm in animals (Fig. 5). The protein is present at the highest level in protein homogenate from *fem-3*gf adults, which produce sperm throughout adulthood (Barton et al., 1987), present at a lower level in protein homogenate from wild-type adult hermaphrodites, which contain spermatozoa, and undetectable in protein homogenate from *fem-2*lf adults, which do not contain any sperm (Kimble et al., 1984).

SPE-11 is segregated to sperm

To determine the localization of SPE-11, affinity purified sera were used for immunofluorescence microscopy. The data shown in Fig. 6 are representative of all three purified antisera. The specificity of the staining pattern shown is demonstrated by the lack of any detectable immunofluorescence staining in *spe-11(hc90)* male gonads (data not shown).

During spermatogenesis, SPE-11 undergoes a series of subcellular relocalizations (Fig. 6). It is first detectable as dots in the nuclei of primary spermatocytes (Fig. 6A,D). The dots appear to coalesce and eventually move to the outside of the condensed DNA, forming a fenestrated ring around the DNA (Fig. 6A,C,D). As discrete chromosomes become visible just prior to the first meiotic division, the protein redistributes throughout the nucleus and sometimes appears to be concentrated between chromosomes (Fig. 6C). Double labeling of primary spermatocytes with antisera to SPE-11 and a monoclonal antibody to nuclear pore complex (NPC) proteins demonstrates that this dynamic series of relocalizations occurs within the nucleus (Fig. 6D). The first and second meiotic divisions occur rapidly in a narrow region of the gonad (Klass et al., 1976). During these divisions SPE-11 is diffuse throughout the cells (Fig. 6A, bracket). After the second meiotic division, two or four spermatids remain attached to a single residual body. In this structure, SPE-11 is localized in a ring around the highly condensed spermatid

A

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   1  gtcgacagaacatttttccgtcaaccggcaaataactactattacagtta
  51  tcctggatacaattattatcaaggaaagaagagaaagtagactatttgga
 101  cgattattttgttcacgaaagaatattatcaataaaaaatgttcaattta
 151  atgtttttgtaataaaacaacgtgacacccttatgaacttaaagttcata
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                          M  S  D  E  E  I  D  I  S
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      T  A  L  N  N  K  T  T  P  K  K  K  S
 351  cagaagtagtcgtctcggcagaggttgacatcatttctgacaatggatat
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                                                      L
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      K  R  N  S  N  S  Q  E  G  Y  E  S  P  E  E  R  E
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       I  V  Y  P  S  V  F  G  A  I  G  T  P  M  A  K  S
 701  ggataacgcaaaagaatgggacgaatggaaagaaaaagaacgaaaaaag
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 801  ggccacttgccgttggtttccgattcagagttttgaaaggaagaaagga
       G  H  L  P  L  V  S  D  S  E  F  L  K  G  R  K  E
 851  gcacaaagagtacaattcaaaagctcgcatggacagtgagttaataatta
        H  K  E  Y  N  S  K  A  R  M  D
 901  aatagttaatggaaattttaaaccattttcagtacttgatggcctggatg
                                      I  L  D  G  L  D
 951  aggttaatgagggattcttcaactgcgggaaaggagcggctatgaatatt
      E  V  N  E  G  F  F  N  C  G  K  G  A  A  M  N  I
1001  cgttataatgataaaaatgtttcaaagaagggagcaaagaagtttgtagc
       R  Y  N  D  K  N  V  S  K  K  G  A  K  K  F  V  A
1051  tactgttggtaagtgtaatctctggtgaataacgtcaaaagtgcaattgc
        T  V
1101  tagtaagacaaaactaacgttaaatggaggatcggatttgtggatctgct
1151  aaattcgatgccataacaaaaaatgtctagaagagtcctaaaccgaattt
1201  ccccagtgaatatgcaaaaaaatttttcagaaaccgcaatgaagaaagca
                                    E  T  A  M  K  K  A
1251  ggtaacccgacaatggagcagatgatgacggatgatttggatgaagatga
       G  N  P  T  M  E  Q  M  M  T  D  D  L  D  E  D  E
1301  agctcgtgctgaagccgaatgggaacgacaacgtgaacagagaaagttag
        A  R  A  E  A  E  W  E  R  Q  R  E  Q  R  K  L
1351  cttcaagagcttatgatgctgcgatggatgagcgtgtatgatatactatc
      A  S  R  A  Y  D  A  A  M  D  E  R
1401  tacaaatttccatttaaatttcaaaattacaggaggacgatgcaaagtat
                                      E  D  D  A  K  Y
1451  gtaccttgggacgagtactgtcaagagatggaggagcttggaaaagaatt
       V  P  W  D  E  Y  C  Q  E  M  E  E  L  G  K  E  L
1501  gaagattggagaaaagcattacaaaaaggtttaactttgaaatactatac
        K  I  G  E  K  H  Y  K  K
1551  actgttgtaatgttttattatagtggcttgaaaagaagatggatgagaat
                                W  L  E  K  K  M  D  E  N
1601  aaggttactcacaaattcaatgcatatcagttggatctcaagtgtctcga
       K  V  T  H  K  F  N  A  Y  Q  L  D  L  K  C  L  D
1651  tgaggacgcttttccaataagtgagtttggagaaatgatgaaaatgttt
        E  D  A  F  S  N  K
1701  aacattaaaaatttcagaaaatcattgaaaagtgttgtgagaaacgttca
                        K  S  L  K  S  V  V  R  N  V  Q
1751  aaagttttatagaaagatgcgtgagccgaagaagtgactcgtgatgatta
        K  F  Y  R  K  M  R  E  P  K  K
1801  gcttactcgtgaaatattgaactatagaaagagcaataaagcttattcga
1851  atacttacaatattattctaaaccaaatgcatttcaacaatcaatatttc
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1951  gttgatcgatatcgagtgcaaattcagaattaactagagat
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B

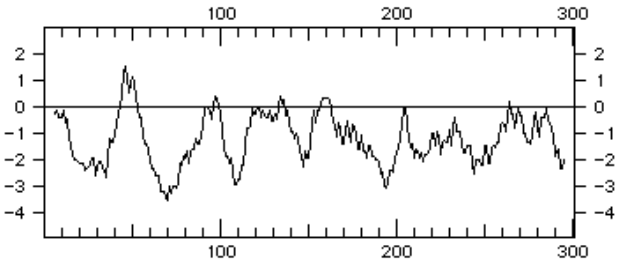


Fig. 3. Sequence analysis of the *spe-11* gene. (A) *spe-11* gene sequence. The genomic and corresponding cDNA (bold type) sequences of the *spe-11* gene are shown. The genomic sequences shown include all the 5′ sequences necessary for rescue of the mutant phenotype. There is a potential TATA box (double underline) beginning 60 bp upstream of the 5′ most nucleotide of the cDNA and a polyadenylation signal sequence (double underline) 3′ of the coding sequence. The predicted amino acid sequence of the protein is shown below the DNA. Potential NLS sequences are underlined. The GenBank accession number is U36483. (B) Kyte-Doolittle hydropathy plot. A Kyte-Doolittle hydropathy plot of SPE-11 drawn by the DNA Strider program illustrates that most of the protein is hydrophilic (Kyte and Doolittle, 1982; Marck, 1988).

nuclei, with some protein often detectable in the residual body (Fig. 6B and inset). The perinuclear ring in spermatids and spermatozoa is not perfectly uniform but instead is characterized by one or more regions of higher SPE-11 concentration (Figs 6B, 7).

SPE-11 is not detectable in the newly fertilized embryo

The fate of SPE-11 after fertilization was examined by immunofluorescence microscopy. Fig. 7 shows a newly fertilized embryo still in the spermatheca. The maternal nucleus (large arrow) has not yet completed the first meiotic division, and the spermatozoan nucleus (small arrow) is still almost fully condensed. Although SPE-11 is perinuclear in the spermatozoa surrounding the embryo (arrowhead), the protein is no longer detectable around the nucleus of the sperm that has fertilized the embryo and is not detectable anywhere else in the embryo. Unless highly localized, the small amount of SPE-11 delivered by the sperm would be difficult to detect in the comparatively large embryo. SPE-11 is also not detectable in later stage embryos (data not shown).

Ectopically expressed SPE-11 localizes to the nucleus

To determine if the nuclear localization of SPE-11 seen during spermatogenesis is specific to the germline or is a general property of the protein, we ectopically expressed SPE-11 under the control of the *C. elegans* heat shock promoter 16 (Stringham et al., 1992). Wild-type hermaphrodites were transformed with an hsp16::*spe-11*cDNA construct, along with a marker for transformation (Mello et al., 1991), and heritable lines were established. Gravid hermaphrodites were heat shocked, then assayed for SPE-11 expression by immunofluorescence microscopy. Heat-shocked embryos displayed cell cycle regulation of SPE-11 localization similar to that seen during spermatogenesis (Fig. 8): in interphase embryonic cells, SPE-11 was concentrated in the nucleus, while in dividing cells, SPE-11 was diffusely distributed throughout the cell. In terminally differentiated cells in which the construct was expressed, SPE-11 was localized to the nucleus (data not shown). The intestine is a notable exception; in this tissue SPE-11 was distributed throughout the cell (data not shown). These results demonstrate that nuclear localization of SPE-11 is not specific to spermatogenesis. However, localization of SPE-11 to discrete regions of the nucleus does appear to be specific to spermatogenesis. The pattern of subnuclear localization observed in primary spermatocytes may require proteins or cellular architecture specific to

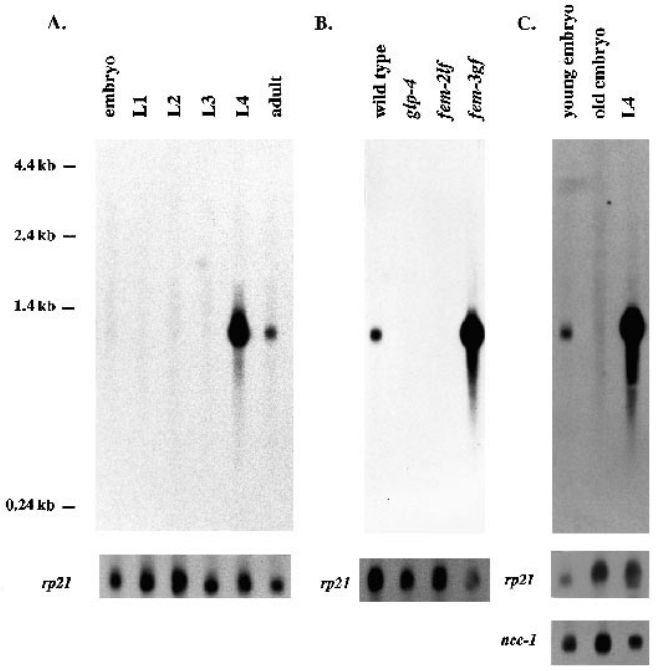


Fig. 4. Transcriptional analysis of the *spe-11* gene. For each strain or developmental stage, approximately 5 μg of poly(A)$^+$ RNA was size-fractionated on a 1.2% agarose 6% formaldehyde gel. The RNA was transferred to Hybond and probed with the *spe-11* cDNA, which hybridizes to an approx. 1.2 kb transcript. The *spe-11* probe was removed, and the blots were reprobed with the ribosomal protein 21 gene, *rp21*, which serves as a loading control (except in C). (A) Developmental northern blot analysis. RNA was isolated from wild-type hermaphrodites at the following developmental stages: embryonic; L1 (first larval); L2 (second larval); L3 (third larval); L4 (fourth larval); adult. (B) Mutant northern blot analysis. RNA was isolated from synchronous adult populations: wild-type adults (contain sperm and oocytes), *glp-4* adults (contain a severely reduced and undifferentiated germline), *fem-2*lf adults (contain only oocytes), *fem-3*gf adults (contain only sperm). Mutant strains were grown at the restrictive temperature. (C) Embryonic northern blot analysis. RNA was isolated from young embryos and older embryos, collected by preparing embryos from young adults and adults, respectively. Although both populations of animals contain a range of embryonic stages, young adults contain a higher proportion of young embryos than adults contain. The young embryo population contained 65% pre-30-cell embryos, and all embryos staged were pre-comma (less than 550-cells) (n=40). The old embryo population contained a mixed population of embryos with some comma and later stage embryos (the exact population distribution was not determined). The relative age of the embryos is further reflected by the ratios of *rp21* mRNA and *ncc-1* mRNA in the two populations of embryos. By in situ hybridization *rp21* mRNA is first detectable at approximately the 550-cell stage, whereas *ncc-1* mRNA is detectable until the 550-cell stage (Seydoux and Fire, 1994; Waterston et al., 1992). By PhosphorImager analysis, the ratio of *rp21* in the old embryo population versus the young embryo population is 4.1:1, while the ratio of *ncc-1* in the old embryo population versus the young embryo population is 2.3:1.

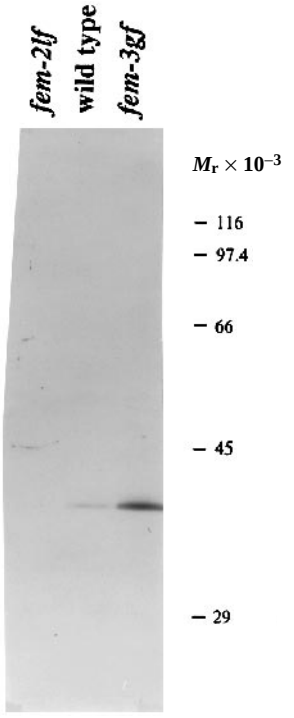
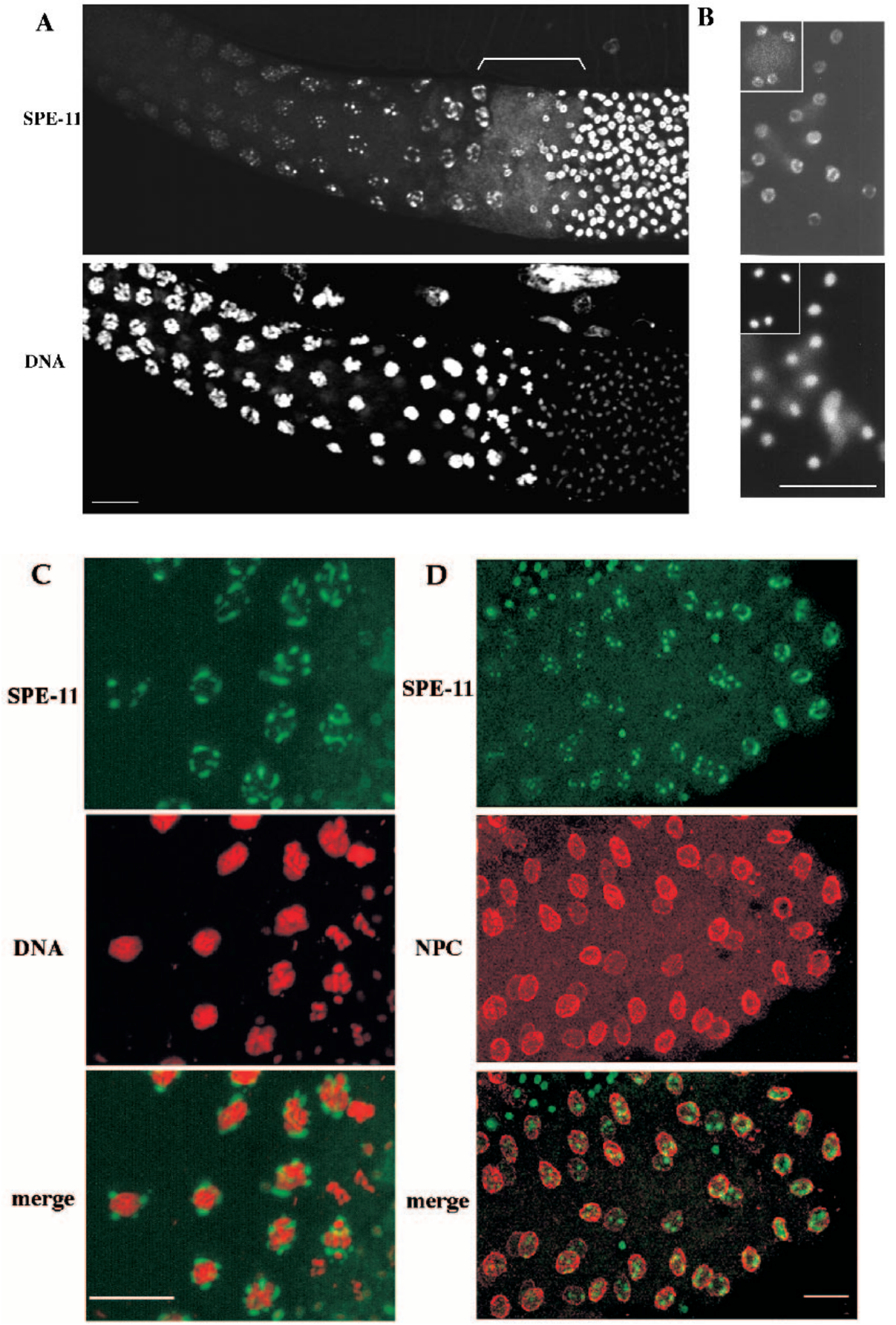


Fig. 5. Western blot analysis. Protein homogenates were obtained from synchronous populations of adult wild-type hermaphrodites (containing both sperm and oocytes), *fem-2*lf animals (containing only oocytes), and *fem-3*gf animals (containing only sperm). Protein was size-fractionated on a 10% polyacrylamide SDS gel and transferred to a nitrocellulose membrane. On the basis of Ponceau S staining of the membrane, approximately equivalent amounts of protein were loaded in each lane (data not shown). The blot was reacted with affinity purified anti-SPE-11 serum from rat 1.

Fig. 6. Immunofluorescence analyses of SPE-11 localization during spermatogenesis. In each confocal micrograph (A,C,D) spermatogenesis proceeds from left to right. SPE-11 is stained with affinity purified antiserum to SPE-11, DNA is stained with propidium iodide or 4′, 6 diamidino-2-phenylindole (DAPI), and nuclear envelopes are stained with a monoclonal antibody (mAb414) to nuclear pore complex (NPC) proteins (Davis and Blobel, 1986). Scale bar, 10 μm. (A) Confocal images of a wild-type male gonad double labeled with antiserum to SPE-11 and propidium iodide. Each micrograph is the projection of a Z series containing seven sequential focal planes including approximately half the gonad thickness. SPE-11 first appears as dots in the nuclei of primary spermatocytes. The dots appear to coalesce before the first meiotic division. During the first and second meiotic divisions, SPE-11 is diffuse throughout the cells (bracket). SPE-11 is localized in a ring around the nuclei of sperm. (B) Conventional epifluorescence micrographs of sperm double labeled with antiserum to SPE-11 and DAPI. SPE-11 is perinuclear in sperm. Inset: Four spermatids attached to a single residual body. Some staining is detectable in the residual body. (C) Confocal images of primary spermatocytes double labeled with antiserum to SPE-11 and propidium iodide. The micrographs are projections of a Z series that includes the entire depth of the tissue. As the DNA becomes condensed in preparation for the first meiotic division, SPE-11 forms a fenestrated ring around the DNA. As discrete chromosomes become visible, SPE-11 relocalizes throughout the nucleus and is sometimes localized between the chromosomes. (This is also evident from analysis of individual focal planes; data not shown.) (D) Confocal images of primary spermatocytes double labeled with antiserum to SPE-11 and NPC antibodies. The micrographs are projections of a Z series that include the entire depth of the tissue. A merge of the NPC and SPE-11 projections demonstrates that the dynamic relocalization that SPE-11 undergoes in primary spermatocytes occurs within the nucleus. Spermatogenesis proceeds from left to right, with the exception of a few sperm (small rings stained with antiserum to SPE-11) in the upper left portion of the micrographs. There is no detectable staining of these sperm with the NPC antibody.


A
SPE-11
DNA
B
C
SPE-11
DNA
merge
D
SPE-11
NPC
merge

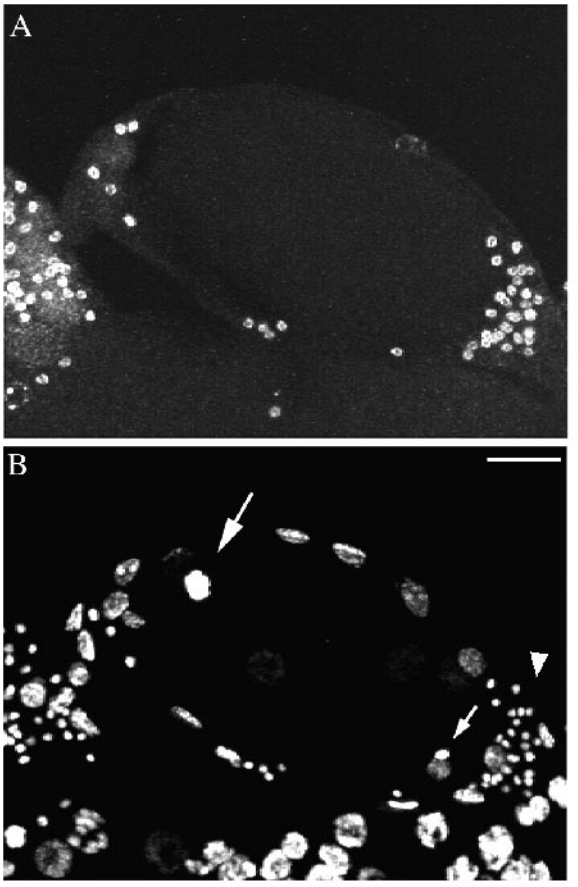


Fig. 7. Analysis of SPE-11 in embryos. The confocal micrographs are projections of a Z series that includes the entire depth of a very early embryo. (A) Immunofluorescence staining of SPE-11. (B) Propidium iodide staining of DNA. The maternal nucleus (large arrow in B) has not completed the first meiotic division, and the spermatozoan nucleus (small arrow in B) has not fully decondensed. The embryo is still in the spermatheca and is surrounded by sperm, which show the characteristic perinuclear staining. SPE-11 is not detectable in the newly fertilized embryo. On the basis of their position in the spermatheca, the sperm in the lower righthand corner are spermatozoa (arrowhead in B). Scale bar, 10 μm.

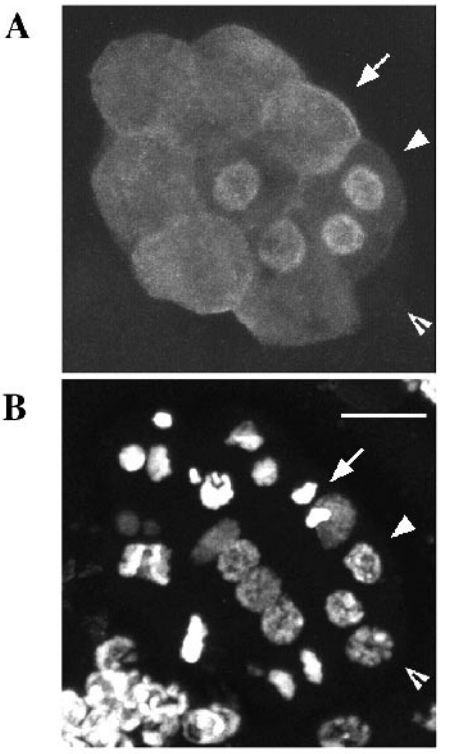


Fig. 8. Ectopic expression of SPE-11 in embryos. The confocal micrographs are projections of a Z series. (A) Immunofluorescence staining of SPE-11. (B) Propidium iodide staining of DNA. SPE-11 was ectopically expressed under the control of the hsp16 promoter. After heat shock, SPE-11 localizes to the nucleus of interphase cells (arrowhead indicates example) and is diffuse in mitotic cells (arrow indicates example). SPE-11 is not detectable in all cells (caret indicates example). In the absence of heat shock, little or no SPE-11 is detectable (data not shown). Scale bar, 10 μm.

the primary spermatocyte or meiotic cell. Unfortunately, as previously described (Stringham et al., 1992), expression from the hsp16 promoter was not detectable in the germline or one-cell embryos (data not shown). Thus, we could not determine the cellular location of ectopically expressed SPE-11 in oocytes or one-cell embryos.

The nuclear localization of SPE-11 in primary spermatocytes, as well as its concentration in nuclei when ectopically expressed, indicate that there are likely to be nuclear localization signal (NLS) sequences in the protein. NLS sequences are generally short regions of positively charged residues associated with a helix destabilizing residue such as proline or glycine (Garcia-Bustos et al., 1991; Forbes, 1992). On the basis of these criteria, SPE-11 has five potential NLS sequences (Fig. 3A, underlined).

Does SPE-11 function in the sperm or the embryo?

To address whether SPE-11's essential function is during spermatogenesis or embryogenesis, we genetically engineered animals to supply SPE-11 through the oocyte rather than the sperm. If the *spe-11* gene product functions directly in the embryo, oocyte expression might be sufficient for embryonic viability. The *spe-11* cDNA, under the control of the heat shock promoter, was injected into the syncytial gonad of adult *spe-11(hc90)dpy-5(e61)* hermaphrodites (Fig. 9). In the adult hermaphrodite this region is committed to oogenesis (Hirsh et al., 1976). The injected hermaphrodites were heat shocked to induce expression from the heat shock promoter in the ovaries. *spe-11*-expressing oocytes were fertilized by mutant sperm in the spermatheca. Injected and heat-shocked hermaphrodites became capable of bearing viable progeny, producing an average of 10 viable progeny per injected gonad arm (Fig. 9A). This suggests that a single burst of *spe-11* expression in the ovary provides about ten oocytes with sufficient levels of SPE-11 for rescue of the embryonic-lethal phenotype.

Several control experiments demonstrated that rescue was dependent on expression of the *spe-11* cDNA (Fig. 9A). First, rescue was heat shock dependent: in the presence of heat shock, 26/29 animals injected with phsp16::*spe-11*cDNA produced viable progeny, whereas in the absence of heat shock, one of 36 animals produced one viable offspring. The production of one offspring in the absence of heat shock was likely due to either low, basal level expression from the promoter or

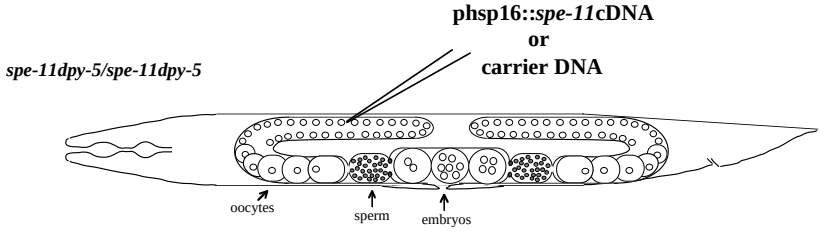
unintentional stress on the animal, resulting in induction of the heat shock promoter. Second, heat shock itself did not rescue the mutant phenotype. Third, injection of unrelated DNA did not result in the production of viable progeny. We were not able to detect SPE-11 by immunofluorescence staining of embryos rescued by maternal expression of *spe-11* (data not shown). Thus, the level of expression from the heat shock promoter in the ovary is sufficient for rescue but not sufficient to be detected in rescued embryos.

To verify that maternally supplied SPE-11 is responsible for rescue in these experiments, we showed that sperm from uninjected *spe-11* males could produce viable progeny when mated to injected and heat-shocked *spe-11* hermaphrodites. *spe-11(hc90)dpy-5(e61)* hermaphrodites were injected with phsp16::*spe-11*cDNA, heat shocked, and then mated to *spe-11(hc90)dpy-5(e61)/spe-11(hc90)+* males. Several days later, broods were scored for the presence of non-Dpy progeny, which resulted from fertilization by male sperm. Five of the seven animals that produced progeny, produced some non-Dpy progeny (Fig. 9B).

Ectopic expression of the *spe-11* gene in the ovary also rescued the mutant phenotype of the two other severe alleles of *spe-11* (data not shown). Thus, oocyte rescue is not dependent on a special characteristic of the *hc90* allele. In fact, the *hc90* allele is most likely a null allele: by genetic criteria *hc90* is a null allele or a severe hypomorph (Hill et al., 1989), the *hc90* allele contains a mutation that results in the introduction of a stop codon at amino acid 191, and SPE-11 is undetectable in *spe-11(hc90)* animals by antibody staining (data not shown).

We also performed these experiments with a heritable line. Heritably transformed *spe-11(hc90)dpy-5(e61)* hermaphrodites carrying phsp16::*spe-11*cDNA that were heated shocked as adults and mated to *spe-11(hc90)* males were capable of producing viable self-progeny and outcross progeny. Eight of 24 heat-shocked hermaphrodites produced viable progeny. (Because extra-chromosomal arrays are lost at variable frequencies from generation to generation, some or all of the animals that did not produce progeny may not have carried the array). Of these eight, three produced viable outcross progeny. In addition, the heritable line was maintained for several months by heat shocking the animals as young adults every generation and allowing them to produce self-progeny. The production of progeny supports the conclusion that a low level of expression from the heat shock promoter is occurring in the germline, although we (previous section) and others (Stringham et al., 1992) have not been able to detect it.

These results demonstrate that ectopic expression in the oocyte is sufficient to rescue the Spe-11 paternal-effect phenotype. Therefore, SPE-11 function is not required during spermatogenesis. This suggests that SPE-11 serves its essential role at fertilization or during early embryogenesis. Thus, in contrast to the many maternally supplied proteins required for



A. ***spe-11dpy-5*** **hermaphrodites**

DNA	Heat Shock	number of fertile animals / number of animals injected
phsp16::*spe-11*cDNA	+	26/29*
	-	1/36**
uninjected	+	0/20
carrier DNA	+	0/7
	-	0/7

B. ***spe-11dpy-5*** **hermaphrodites mated to** ***spe-11dpy-5/spe-11+*** **males**

DNA	Heat Shock	number of fertile animals / number of animals injected	number of animals that produced outcross progeny / number of fertile animals
phsp16::*spe-11*cDNA	+	7/11	5/7
	-	0/11	
uninjected	+	0/13	

Fig. 9. Oocyte rescue experiment. The syncytial ovaries of adult *spe-11(hc90)dpy-5(e61)* hermaphrodites were injected with the specified DNA. Some animals were heat shocked for two hours at 33-35°C. Several days after heat shock, the production of viable progeny was assessed. Animals that produced viable progeny were scored as fertile. Control unmated *dpy-5* hermaphrodites produce an average of 38 progeny per gonad arm (n=10); if mated, they can produce many more progeny. (A) Injected and control animals were allowed to produce self-progeny. *For ten of these injected and heat-shocked animals, a single gonad arm was injected and the number of viable progeny produced was counted. They produced an average of ten progeny per injected arm (range 0 to 21). **One of 36 non-heat-shocked hermaphrodites injected with phsp16::*spe-11*cDNA produced one offspring. (B) Injected and control *spe-11(hc90)dpy-5(61)* hermaphrodites were mated to *spe-11(hc90)dpy-5(e61)/spe-11(hc90)+* males. The production of outcross progeny (progeny resulting from fertilization by male sperm) was assessed by the presence of non-Dpy progeny.

embryogenesis, SPE-11 appears to be a paternally supplied protein required for embryogenesis.

DISCUSSION

SPE-11 functions in the early embryo

Phenotypic analysis of the paternal-effect embryonic-lethal gene *spe-11* demonstrated that sperm from *spe-11* animals are capable of entering oocytes, but that the resulting embryos develop abnormally (Hill et al., 1989). We have shown here that SPE-11 is synthesized during spermatogenesis and is localized to the perinuclear region of mature sperm. At or shortly after fertilization it is no longer detectable around the sperm nucleus and is not detectable anywhere else in the embryo. These observations led us to investigate whether SPE-11's role in early embryogenesis is indirect or direct. SPE-11 could function during spermatogenesis to make the sperm competent to initiate correctly the program of early embryogenesis, for example by properly assembling the perinuclear halo in immature sperm. Alternatively, SPE-11 could be delivered to the embryo by the sperm and function directly in the early events of embryogenesis. To address this question, the gene product was supplied to the embryo through the oocyte rather than through the sperm. Some of the resulting embryos developed normally. This result demonstrates that SPE-11 is not required for spermatogenesis. More importantly, the finding that SPE-11 can be supplied through either gamete suggests that it functions at or after fertilization and therefore has a direct role in embryogenesis.

The results of temperature-shift experiments further support this conclusion by demonstrating that SPE-11 function during spermatogenesis is not sufficient for embryonic viability: hermaphrodites that were mated to *spe-11(hc77*ts) males at the permissive temperature and then shifted to the restrictive temperature produced some inviable embryos (Hill et al., 1989). If SPE-11 functions only during spermatogenesis, then its function would have been completed before the shift and all the embryos would have developed normally. This result argues against two alternative interpretations of the oocyte rescue experiment: (1) SPE-11 normally functions during spermatogenesis, but if ectopically expressed in oocytes, it serves that same role during oogenesis. (2) SPE-11 normally functions during spermatogenesis, but in the oocyte rescue experiment, it performs that same role after fertilization (i.e. it acts on the sperm or a sperm component inside the embryo). Although unlikely, it remains a possibility that SPE-11 normally functions in mature sperm, but in the oocyte rescue experiment it executes that same role in the embryo. The leaky nature of the *hc77* allele precludes performing the reciprocal downshift experiment to examine whether SPE-11 function in the embryo is sufficient for embryonic viability (Hill et al., 1989). However, the oocyte rescue results presented here address that issue, and taken together with the temperature-shift results, support the hypothesis that SPE-11 functions in the early embryo.

Properties of SPE-11

SPE-11 is not significantly similar to previously identified proteins. However, the protein does have several notable characteristics. It is very hydrophilic: 41% of the residues are acidic or basic amino acids. Although rich in charged residues, the number of acidic and basic residues is nearly equal (61 and 62, respectively) and the isoelectric point of the protein is calculated to be 7.93.

SPE-11 localizes to the nucleus of primary spermatocytes in wild-type males and hermaphrodites. This ability to localize to the nucleus is not limited to primary spermatocytes; SPE-11 also localizes to the nucleus of most cells in which it is ectopically expressed. In accordance with these observations, the protein contains five potential NLS sequences. Unfortunately, we could not detect either the endogenously expressed SPE-11 or ectopically expressed SPE-11 in one-cell embryos, where we think SPE-11 performs its essential role. However, nuclear localization is probably not important for SPE-11's function in the newly fertilized egg, because the earliest defects observed in Spe-11 embryos occur before nuclear envelope formation. The oocyte nuclear envelope appears to break down shortly before fertilization (Ward and Carrel, 1979) and does not reform until after the completion of the second meiotic division (D. Albertson, personal communication). The sperm nuclear envelope is not detectable after the second meiotic division of spermatogenesis, and both spermatids and spermatozoa probably lack a nuclear envelope (Wolf et al., 1978; Kimble and Ward, 1988; Fig. 6D). Nuclear envelopes appear for the first time in the one-cell embryo as the maternal and paternal pronuclei form. By this time, Spe-11 embryos already display their earliest defects: failure to complete meiosis and failure to form a hard eggshell (Hill et al., 1989). Nuclear localization could be important for function if SPE-11 is directly involved in some of the later events that are defective in one-cell Spe-11 embryos or if SPE-11 functions in later stage embryos.

Transcription of *spe-11*

As expected from its paternal-effect phenotype, the *spe-11* gene is expressed in animals undergoing spermatogenesis and is not detectable in females undergoing oogenesis. The transcript is also detectable in early embryos. This could be the result of transcription of *spe-11* in the embryo. However, based on its detectability in young but not old embryos and the relatively high level of transcription during spermatogenesis, we think the most likely source of this embryonic transcript is the sperm. Genetic evidence also indicates that embryonic transcription is neither necessary nor sufficient for embryonic viability, since homozygous mutant embryos develop normally so long as their father was heterozygous, and heterozygous embryos develop abnormally if their father was homozygous mutant (Hill et al., 1989).

There are several possible explanations for the presence of the *spe-11* transcript in embryos. The mRNA may serve a structural role in the sperm, possibly in the perinuclear electron-dense halo, and may end up in embryos by virtue of its association with this sperm structure. Consistent with this possibility, the halo is thought to contain RNA (Ward et al., 1981). A second possibility is that a small amount of the transcript remains in the sperm simply because it is relatively stable and is not segregated to the residual body. A third possibility is that the transcript is delivered to the embryo for translation. If this is the case, translation does not result in detectable levels of protein.

Perinuclear localization of SPE-11 in sperm

The spermatid is a streamlined cell, having left behind in the residual body many cellular components. It has been suggested that components that must be segregated to the spermatid may require special localization mechanisms (Ward, 1986). Some proteins destined for the spermatid are packaged into special, transient structures, the fibrous body-membranous organelle complexes (Roberts et al., 1986). Similarly, SPE-11's perinuclear localization in the spermatid may serve as a mechanism to guarantee that the protein is segregated to the spermatid and ultimately delivered to the embryo.

Although SPE-11 is not required for spermatogenesis, it does perform a nonessential role in the spermatid and spermatozoan. In sperm from animals bearing the three severe alleles of *spe-11*, the electron-dense perinuclear halo is unevenly distributed and contains extra granular material, indicating that SPE-11 is required for normal halo morphology (Hill et al., 1989, and H. B., C. Sganga, F. R. Turner and S. S., unpublished result). Furthermore, SPE-11's perinuclear localization in sperm suggests that the protein is a component of the halo.

Possible roles for SPE-11 in embryogenesis

What role does SPE-11 play in embryogenesis? Known paternal contributions to the embryo include a signal for activation of the oocyte, a centrosome (which can be involved in the establishment of an embryonic axis), and a haploid genome. SPE-11 does not appear to be a centrosomal component: in Spe-11 embryos the sperm-supplied centrosomes nucleate normal appearing arrays of microtubules, and pronuclear migration and mitosis occur apparently normally (Hill et al., 1989). Furthermore, SPE-11 is not localized to the centrosome. SPE-11 also does not appear to be involved in establishment of the embryonic axis: the defects seen in Spe-11 embryos are more pleiotropic than would be expected from an absence of polarity. SPE-11 is not required for delivery of the sperm-contributed haploid genome, since Spe-11 embryos contain a paternal nucleus. Furthermore, the nucleus must contain a normal complement of chromosomes, because it can support normal development if SPE-11 is ectopically provided via the oocyte.

The early and numerous defects displayed by Spe-11 embryos suggest that these embryos have not been correctly or completely activated (Hill et al., 1989). Thus, SPE-11 may be involved in oocyte activation. However, SPE-11 alone does not activate the oocyte: oocytes in which the *spe-11* gene was ectopically expressed remained competent for fertilization (Fig. 9), and expression of the *spe-11* gene in spermless hermaphrodites did not result in the production of oocytes with visible eggshells (H. B., unpublished observation). In fact, Spe-11 embryos are partly activated: they show a block to polyspermy, form a weak eggshell, undergo pronuclear migration, undergo an aberrant pseudocleavage, and undergo mitosis (Hill et al., 1989). Consequently, there must be other sperm-supplied components necessary for activation. One possibility is that SPE-11 and other components of the perinuclear halo in sperm are required to initiate the embryonic program. The observation that SPE-11 is no longer detectable around the sperm nucleus at or shortly after fertilization may indicate the disassembly of the halo and the dispersal of halo components for interaction with oocyte targets. If the oocyte targets are broadly distributed, then the relatively small amount of SPE-11 delivered by the sperm would be likely to fall below the limits of detectability.

Why is SPE-11 delivered to the embryo via the sperm?

If, as we propose, SPE-11 functions in the embryo, why is it delivered via the sperm rather than the oocyte? *C. elegans* sperm are small compared to oocytes (Hirsh et al., 1976) and based on our calculations contribute less than 1% of the oocyte volume to the zygote. Consequently, unless there is an advantage to sperm delivery, one would expect that most components necessary for embryogenesis would be provided by the oocyte. There are at least three reasons why it may be beneficial to supply SPE-11 via the sperm: (1) If SPE-11 is involved in oocyte activation, it may be preferable to store it (along with other activation components) in the sperm rather than the oocyte, so that the oocyte is not prematurely or accidentally activated. (2) SPE-11 also functions in the sperm. Although SPE-11's involvement in the organization of the electron-dense perinuclear material in the sperm is not required, a sperm that has a 'good' halo may be superior to a sperm with an aberrant halo. (3) Once in the embryo, SPE-11 may act on or in conjunction with another sperm-supplied component. Thus, SPE-11 may be delivered by the sperm so that it is in close proximity to another sperm component. Further analysis of SPE-11's function and identification of the proteins with which it interacts in the sperm and embryo may reveal why it is supplied by the sperm.

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