A Sperm-Supplied Product Essential for Initiation of Normal Embryogenesis in Caenorhabditis elegans Is Encoded by the Paternal-Effect Embryonic-Lethal Gene, spe-11

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Loss-of-function mutations in the spe-11 gene in Caenorhabditis elegans result in a paternal-effect embryonic-lethal phenotype: fertilization of wild-type oocytes by sperm from homozygous spe-11 mutant males leads to abnormal zygotic development, whereas oocytes from homozygous spe-11 hermaphrodites when fertilized by wild-type sperm develop normally. Embryos fertilized by sperm from homozygous spe-11 worms fail to complete meiosis and show defects in eggshell formation, mitotic spindle orientation, and cytokinesis. Genetic analysis suggests that the spe-11 gene is expressed before the completion of spermatogenesis and that the wild-type locus encodes a product that is present in sperm and participates, directly or indirectly, in initiating the correct program of early events in C. elegans embryos. Such an autogenetic role of the spe-11 gene product in early embryogenesis distinguishes spe-11 mutations from the two paternal-effect mutations identified in Drosophila, ms(2)K81 and pol, which primarily affect chromosome behavior.


INTRODUCTION

In a wide variety of organisms, early embryonic development is guided primarily by maternal components. For example, eggs from sea urchins, starfish, annelids, molluscs, and frogs can be parthenogenetically activated (for review, see Wilson, 1925), and in many cases early development of these fatherless embryos proceeds normally, demonstrating the absence of a requirement for sperm entry or a paternal genome (Shearer and Lloyd, 1912; Loeb, 1913; Tyler, 1931). Further evidence for the importance of maternal components comes from the existence of large collections of maternal-effect embryonic-lethal mutations in Drosophila and Caenorhabditis elegans (Nusslein-Volhard and Weischaus, 1980; Perrimon et al., 1986; Kemphues, 1987; Kemphues et al., 1988); such mutations identify genes whose products must be provided in the oocyte for normal embryonic development. Furthermore, transcription of the embryonic genome in most organisms is not globally activated until many divisions after fertilization (Hecht et al., 1981; Newport and Kirschner, 1982; Edgar and Schubiger, 1986); the oocytes of these organisms contain large stockpiles of proteins and mRNAs to support early development.

Although these observations suggest that control of early embryogenesis is mainly maternal, the sperm serves several critical and often essential roles in early development: (1) Sperm entry activates the oocyte and initiates the program of early events seen in the zygote. On the basis of measurements of intracellular Ca$^{2+}$ levels (reviewed by Jaffe, 1983) and the activation of oocytes by the ionophore A23187 (Steinhardt et al., 1974), the mechanism of activation in many organisms appears to involve a sperm-induced rise in intracellular free calcium ions. (2) The sperm contributes a haploid genome to the haploid oocyte, to generate a diploid zygote. In mouse embryos, paternally imprinted chromosomes are essential for development of the trophoblast (Surani et al., 1984; McGrath and Solter, 1984). (3) In many organisms, the sperm contributes a microtubule-organizing center. In C. elegans, sea urchin, and ascidian embryos, the sperm-contributed microtubule-organizing center nucleates growth of the first mitotic spindle (Albertson, 1984; Chambers, 1939; Sawada and Schatten, personal communication). (4) In some organisms, sperm entry appears to participate in establishment of embryonic axes. In C. elegans, the site of sperm entry...
may specify the anterior-posterior axis (Albertson, 1984; Schierenberg, 1986), and in *Xenopus*, the dorsal-ventral axis is normally determined by the site of sperm entry (Gerhart, 1989). The sperm functions mentioned above have been deduced by identifying visible components that the sperm contributes to the zygote and by analyzing unfertilized oocytes manipulated in various ways, such as by treating with ionophore or by injecting centrosomes or an egg or sperm pronucleus.

We have taken advantage of the genetics and self-fertilization properties of *C. elegans* to identify mutations that impair sperm functions necessary for early embryonic development. *C. elegans* generally reproduces as a hermaphrodite, producing approximately 300 sperm during the fourth larval stage (L4) and then converting from spermatogenesis to oogenesis. Oocytes are continuously produced throughout adulthood. If never mated, hermaphrodites produce exclusively "self" progeny. As a result of X chromosome nondisjunction, XX hermaphrodites produce approximately 0.15% XO male progeny. After mating hermaphrodites with males, oocytes are preferentially fertilized by the male-contributed sperm, resulting in the production of "outcross" progeny (Ward and Carrel, 1979). Mutations that affect spermatogenesis and/or sperm function can be isolated by screening for self-sterile hermaphrodites that produce offspring upon mating to wild-type males. Most of the spermatogenesis-defective (*spe*) mutants isolated to date produce defective sperm that are unable to fertilize oocytes (L'Hernault et al., 1988). Mutations in the *spe-11* gene are unusual; they do not affect the production or function that is necessary for zygotic events to occur normally. Analysis of paternal-effect lethal mutants provides a means of genetically defining the sperm's contributions to early embryogenesis.

**MATERIALS AND METHODS**

*Maintenance of C. elegans.* Worms were grown and maintained at 16°C on agar plates with *Escherichia coli* strain OP50 as a food source (Brenner, 1974). The following chromosome I genes and mutations were used: *spe-11* (*hc90* and *hc77ts*) (L'Hernault et al., 1988), *spe-8* (*hc53*) (L'Hernault et al., 1988), *dpy-5* (*e61*), the free duplication *sDp2* (Rose et al., 1984), and the deficiency *sDf3* (Rose, 1980). *spe-11* maps <0.1 map units to the left of *dpy-5* (L'Hernault et al., 1988; A. Rose, personal communication) and is under *sDf4* and covered by *sDp2* (L'Hernault et al., 1988). Genetic crosses were performed as described by Brenner (1974). The permissive and restrictive temperatures for *spe-11* (*hc77ts*) are 16 and 25°C, respectively.

**Immunofluorescent staining.** Fixation and staining were performed as described in Strome and Wood (1982). The DNA stain diamidinophenyl indole dihydrochloride (DAPI; Boehringer-Mannheim) was used to stain embryonic nuclei. To stain DNA in whole worms, the worms were quick frozen in liquid N₂, fixed in 3:1 ethanol:acetic acid at 0°C for 30 min, and then incubated with 0.5 µg/ml DAPI. The worms were then washed with phosphate-buffered saline and mounted on glass slides for viewing. Staining of P granules was carried out using K76, a mouse monoclonal antibody directed against P granules (Strome and Wood, 1983); >100 stained embryos were examined. Staining of sperm-specific antigens was carried out using SP56, a mouse monoclonal antibody directed against *Drosophila melanogaster* α-tubulin (Piperno and Fuller, 1985), and centrosomes were stained with a rabbit preimmune serum (Strome, 1986). Simultaneous staining with more than one antibody (>17 embryos examined) was performed by incubating slides first with one primary antibody, then with the other, and finally with a mixture of the appropriate secondary antibodies.

**Microscopy and photography.** Living embryos were monitored by Nomarski differential interference contrast microscopy using a Zeiss ICM 405 inverted microscope with a 40X planachromat objective. Fluorescence microscopy was performed using epifluorescence optics and a 40X neofluar objective. Embryos were examined with 515-560 nm epillumination for rhodamine, 450-490 nm for fluorescein, and 365 nm for DAPI-stained DNA. Photographs were taken using Kodak Tri-X film at ASA 1600. Film was developed using Diafine two-bath developer (Acufine, Inc., Chicago, IL).

**Transmission electron microscopy.** Virgin adult males, 2 days after their final molt, were cut with a needle below the pharynx in 10 µl of pH 7.0 sperm medium (SM: 50 mM NaCl, 25 mM KCl, 5 mM CaCl₂, 1 mM MgSO₄, and 5 mM Hepes) (Ward et al., 1983) to release the testis. The testes were then fixed by adding 100 µl of 1% formaldehyde, 1% glutaraldehyde in SM. Samples were incubated at room temperature for 1 hr and then overnight at 4°C. The fixed testes were embedded in 1% agar blocks for subsequent handling. The blocks were rinsed in 0.1 M cacodylate buffer, pH 7.4, and fixed in 1% OsO₄ in 0.1 M cacodylate buffer, pH 7.4, for 1 hr. They were then rinsed in H₂O, dehydrated through a series of graded alcohols, and treated with propylene oxide, followed by resin (3:2 Epon Luft:Quetol 651).
RESULTS

spe-11 Is a Paternal-Effect Embryonic-Lethal Gene

Two mutant alleles of the spe-11 gene were obtained in Spe mutant screens (L'Hernault et al., 1988). Hermaphrodites homozygous for the nonconditional allele, hc90, are sterile; they produce sperm and oocytes, but their embryos arrest during early development (Table 1A). Hermaphrodites homozygous for the temperature-sensitive allele, hc77, produce both viable and arrested offspring at the restrictive temperature, as described in more detail in a later section. The results of several different crosses using the nonconditional allele demonstrate that sperm but not oocytes from homozygous spe-11 worms are defective: (1) Mating of homozygous spe-11 hermaphrodites with wild-type males leads to the production of normal outcross embryos (Table 1C). Thus, oocytes from mutant worms can give rise to normal embryos after fertilization by normal sperm, but not after fertilization by sperm produced by mutant worms. (2) Mating of spe-11+ hermaphrodites with homozygous spe-11 males leads to the production of embryos that appear identical to those produced by unmated spe-11 hermaphrodites (Table 1D). Two different spe-11+ hermaphrodites were used in this analysis: dpy-5/dpy-5 hermaphrodites produce only Dumpy self progeny and failed to produce non-Dumpy outcross progeny after they were mated with spe-11 males; spe-8/spe-8 hermaphrodites, which lose their own sperm after laying a few oocytes (L'Hernault et al., 1988), failed to produce normal embryos after mating with spe-11 males. When the embryos produced by the hermaphrodites from these crosses were analyzed, many of the embryos displayed the spe-11 phenotype (see below for description). These results indicate that sperm from homozygous spe-11 worms lead to the production of abnormal embryos. In keeping with using "maternal-effect embryonic-lethal" to describe mutations in oocyte functions and factors that affect embryogenesis, spe-11 is a paternal-effect embryonic-lethal gene.

Mutations in the spe-11 Gene Are Recessive, and the Gene Is Expressed Before the Completion of Meiosis

The recessiveness of the spe-11 mutations was demonstrated by mating males of the genotype spe-11 dpy-5/

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### Table 1

**PROGENY PRODUCED BY spe-11 HERMAPHRODITES AND MALES**

<table>
<thead>
<tr>
<th>Hermaphrodite parent</th>
<th>Male parent</th>
<th>Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. spe-11(hc90)*'</td>
<td>None</td>
<td>Defective embryos*</td>
</tr>
<tr>
<td>spe-11(hc90)</td>
<td>spe-11(hc90)</td>
<td></td>
</tr>
<tr>
<td>B. spe-11(hc90)/sDf4</td>
<td>None</td>
<td>Defective embryos</td>
</tr>
<tr>
<td>C. spe-11(hc90)</td>
<td>+</td>
<td>Normal</td>
</tr>
<tr>
<td>spe-11(hc90)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>D. dpy-5(e61)</td>
<td>spe-11(hc90)</td>
<td>Normal Dumpy self progeny, defective outcross progeny</td>
</tr>
<tr>
<td>dpy-5(e61)</td>
<td>spe-11(hc90)</td>
<td></td>
</tr>
<tr>
<td>spe-8(hc58)</td>
<td>spe-11(hc90)</td>
<td>Defective embryos</td>
</tr>
<tr>
<td>spe-8(hc58)</td>
<td>spe-11(hc90)</td>
<td></td>
</tr>
<tr>
<td>E. spe-11(hc90) dpy-5(e61)</td>
<td>spe-11(hc90) dpy-5(e61)</td>
<td>Normal Dumpy and non-Dumpy progeny*</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>F. spe-11(hc90) dpy-5(e61)</td>
<td>spe-11(hc90) dpy-5(e61)</td>
<td>Normal</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* L'Hernault et al. (1988).

*The number of defective embryos produced cannot be accurately determined. The embryos are generally so fragile that they lyse in the uterus or shortly after they are laid on the plates.

*spe-11 is under the deficiency sDf4.

*spe-8 hermaphrodites have infertile sperm and therefore do not produce self progeny.

*All progeny (81 ± 18 Dumpy and 92 ± 22 non-Dumpy progeny) are outcross.

The free duplication sDp5 covers spe-11 and dpy-5.
Sperm from Homozygous spe-11 Worms Are Fertilization-Competent

Four observations indicate that eggs laid by spe-11(hc90) hermaphrodites, although round and fragile, have been fertilized: (1) They stain positively with a monoclonal antibody that recognizes a sperm-specific antigen (Ward et al., 1986) (Fig. 1). In both wild-type embryos and embryos fertilized by sperm from spe-11 worms, this antigen is seen as a patch at the site of sperm entry and disperses during the first cell cycle (Strome, 1986). (2) They are surrounded by an eggshell, albeit a weak eggshell (Fig. 2b). (3) They contain two pronuclei (Figs. 2b, 2d). (4) They contain a spindle, undergo mitosis, and initiate cytokinesis (Figs. 2f, 2h). None of these four characteristics is observed in the unfertilized eggs produced by spermless hermaphrodites (data not shown).

Embryonic Defects Observed in Eggs Fertilized by Sperm from spe-11(hc90) Worms

The nonconditional allele of spe-11, hc90, displays 100% penetrance and produces severe defects in early embryogenesis. The temperature-sensitive allele, hc77, shows both incomplete penetrance and expressivity at restrictive temperature. This section describes the defects in development seen with the nonconditional allele, hc90. The term “spe-11(hc90) embryos” refers to embryos produced by homozygous spe-11(hc90) hermaphrodites and embryos produced by wild-type hermaphrodites mated with homozygous spe-11(hc90) males; the phenotypes of these embryos are indistinguishable. Embryos from hemizygous hermaphrodites with spe-11(hc90) under a deficiency of the region, sDf4, are also inviable (Table 1B) and display the same defects seen in embryos from hc90 hermaphrodites, suggesting that hc90 is a hypomorphic or null allele (see Discussion).

To determine when and how embryogenesis goes awry in spe-11 embryos, we monitored living embryos by Nomarski microscopy. Wild-type embryos are constrained to their oblong shape by a three-layered eggshell (Fig. 2a), which forms within the first 30 min after fertilization and renders the embryo relatively insensitive to external conditions (see Wood, 1988, for review of early embryogenesis). The earliest defect seen in spe-11(hc90) embryos is a weak eggshell. spe-11(hc90) embryos are round, misshapen, and osmotically sensitive (Fig. 2b). When dissected into hypotonic solutions, they lyse. When dissected into the isotonic culture medium used to culture normal embryos whose eggshells have been disrupted, they are viable for hours, indicating that their round shape is not a result of osmotic swelling.

C. elegans oocytes are arrested at prophase of meiosis I. Wild-type embryos complete meiosis during the first 30 min after fertilization and extrude two polar bodies at the anterior end of the embryo. In spe-11(hc90) embryos, no polar body is visible by Nomarski, suggesting that these embryos do not complete meiosis. To address this issue, we fixed and stained embryos with the DNA-specific stain DAPI during pronuclear migration. This permits the visualization of individual chromosomes in each pronucleus. Normal embryos contain 6 chromosomes in each pronucleus (for example, see Fig. 3a), whereas spe-11(hc90) embryos contained 6 chromosomes in one pronucleus, presumably that from the sperm, and 8–12 discernible chromosomes in the other pronucleus (20 of 21 embryos examined; data not shown). This result indicates that spe-11 embryos fail to undergo either one or both meiotic divisions. To discriminate between these possibilities, we examined emb-
Fig. 3. P-granule distributions in wild-type, spe-11 (hc77ts), and spe-11 (hc90) embryos. Embryos were fixed and stained with DAPI (a, c) and K76, a monoclonal antibody directed against P granules (b, d, e, f). (a, b) A wild-type embryo at pronuclear meeting. The arrow points to the DAPI-stained polar bodies at the anterior end of the embryo; P granules are segregated to the posterior cortex. (c, d) A mutant embryo from an hc77ts hermaphrodite raised at restrictive temperature. The embryo is in telophase of the first cell cycle. The arrow points to the polar bodies; P granules are segregated to the cortex at the opposite end of the embryo. (e, f) Embryos from hc90 hermaphrodites. P granules are localized to a discrete region in each embryo; they appear to be cortically localized in the embryo in f. Bar = 10 μm.

Fig. 2. Nomarski micrographs at progressively later stages of development of wild-type embryos (a, c, e, g, i) and embryos fertilized by sperm from homozygous spe-11 (hc90) worms (b, d, f, h, j). For the wild-type embryos, anterior is left, posterior is right. (a, b) Two pronuclei are visible at opposite ends of each embryo. hc90 embryos do not undergo the regular pseudocleavage contractions seen in wild-type embryos. (c, d) In both wild-type and hc90 embryos the pronuclei meet at one end of the cell. (e, f) In wild-type embryos the spindle is always oriented along the path of pronuclear migration, which corresponds to the anterior–posterior axis. In this hc90 embryo (the same embryo shown in b and d) the spindle is oriented perpendicularly to the path of pronuclear migration. (Arrows indicate spindle poles.) (g, h) The wild-type zygote has divided into a two-cell embryo. An hc90 embryo (not the same embryo shown in b, d, and f) appears to have initiated cytokinesis, which was later aborted. (i, j) The wild-type embryo has divided into a normal four-cell. The later-stage hc90 embryo is unicellular but contains multiple nuclei. Nine of nine hc90 embryos that were monitored continuously displayed the defects shown in this figure. Other embryos monitored for shorter intervals also displayed similar phenotypes. Bar = 10 μm.
Shortly after meiosis, wild-type embryos undergo a number of directed movements of intracellular components that foreshadow their division into two morphologically and developmentally different daughter cells (Nigon, 1960; Albertson, 1984; Strome, 1986). These include a series of contractions of the anterior cell membrane, referred to as pseudocleavage (Figs. 2a, 2c); anterior-to-posterior streaming of the cytoplasm; migration of the egg pronucleus to meet the sperm pronucleus in the posterior end of the embryo (Fig. 2c); and segregation of germ line-specific P granules to the posterior cortex of the embryo (Fig. 3b). Later in the cell cycle, the mitotic spindle moves toward the posterior end of the embryo (Fig. 2e), and the zygote undergoes an unequal division (Fig. 2g), generating blastomeres that differ in both size and developmental fate (Sulston et al., 1983). Most of the events described above occur aberrantly in spe-11(hc90) embryos.

Instead of normal pseudocleavage and cytoplasmic streaming, spe-11(hc90) embryos extend what look like pseudopodial processes in several directions. During this period, one of the pronuclei migrates to meet the other at one end of the embryo (Fig. 2d). We presume it is the egg pronucleus, the same pronucleus that migrates in wild-type embryos. However, since spe-11(hc90) embryos are round and when examined outside of worms do not contain polar bodies, it is not possible to unambiguously identify the anterior or posterior end of the embryo or distinguish the two pronuclei. After pronuclear meeting, the pronuclei migrate to the center of the zygote where a mitotic spindle is established (Fig. 2f).

In wild-type embryos, the mitotic spindle rotates and becomes aligned along the path of pronuclear migration (the anterior–posterior axis of the embryo) (Hyman and White, 1987) (Fig. 2e). In contrast, the mitotic spindle in spe-11(hc90) embryos appears unoriented with respect to the path of pronuclear migration (Fig. 2f), implying that the spindle microtubules are unable to correctly interpret or respond to orientation cues. spe-11(hc90) embryos complete mitosis and initiate cytokinesis (Fig. 2h), but in most cases, this attempted cleavage is aborted and several nuclei form in a single cytoplasm. Since the cleavage furrow position in C. elegans is determined by the position of the mitotic spindle (Albertson, 1984; Hill and Strome, 1988), this defect may be a direct result of the mitotic spindle defect described above. Many rounds of mitosis without cytokinesis lead to the production of unicellular embryos containing many nuclei (Fig. 2i). In a few cases, cytokinesis is successful, and two cells, usually similar in size, are generated; these embryos often fail to undergo cytokinesis in later cell cycles and also generate embryos with multinucleate cells (not shown).

Embryos Fertilized by Sperm from spe-11(hc77ts)
Worms Show Variable Defects in Embryogenesis

Embryos produced by homozygous hc77ts hermaphrodites raised at restrictive temperature range from appearing wild-type and undergoing normal embryogenesis to showing defects as severe as those described above for hc90. The embryos that display phenotypes intermediate between wild-type and hc90 make weak eggshells, but contain at least one polar body. In these intermediate hc77ts embryos, as in hc90 embryos, the mitotic spindle appears unoriented in the first and subsequent cell cycles. However, these embryos often undergo a number of cell divisions and develop into round, multicellular embryos (Fig. 4), implying that the spindle–cortex interactions needed for cleavage furrow formation and cytokinesis are occurring. These embryos do not develop birefringent gut granules, a sign of intestinal differentiation (Lauber et al., 1980), or display any signs of morphogenesis.

The hc77ts embryos that develop apparently normally and hatch are generally produced by young homozygous hc77ts hermaphrodites. Wild-type worms (n = 9) grown at 25.5°C lay viable embryos for 36 hr and produce 105 ± 17 offspring. In contrast, homozygous spe-11(hc77ts) worms (n = 15) grown at 25.5°C lay viable embryos for 7 hr and produce 15 ± 7 offspring. Thus, it appears that as hc77ts hermaphrodites’ sperm age, more hc90-like embryos are produced, as if mutant spe-11 gene product is progressively inactivated or degraded at restrictive temperature.

To determine whether or not the product of the spe-11 gene is likely to be packaged into sperm, we carried out a temperature-upshift experiment. Homozygous spe-11(hc77ts) males were raised at permissive temperature and mated with homozygous dpqy-5 hermaphrodites at permissive temperature for 8 hr. The males were removed from the plates, and the mated hermaphrodites were shifted to restrictive temperature for 19 hr, after which embryos were dissected from their uteri. Nine of the 10 worms examined contained embryos of the hc77ts phenotype, indicating that they had been fertilized by defective sperm. Since the spe-11 gene is transcribed before the completion of meiosis, and since only hc77ts spermatids that had been transferred during mating were exposed to restrictive temperatures, these results indicate that the temperature-sensitive product, probably the spe-11 gene product, is packaged into spermatids. In principle, the reciprocal temperature-downshift experiment should indicate whether the temperature sensitivity of the spe-11 lesion is reversible. However, due to the incomplete penetrance of the hc77ts allele, such an experiment does not yield conclusive results.
Fig. 4. Nomarski micrographs of embryos produced by spe-11 (hc77ts) hermaphrodites grown at restrictive temperature (25.5°C). (a) A two-cell embryo. (b) An eight-cell embryo. Some of the cells contain multiple nuclei. (c) A round multicellular embryo that shows no signs of initiating morphogenesis. Bar = 10 μm.

P Granules Coalesce and Appear to Be Segregated in Some spe-11 Embryos

To assess the ability of spe-11 zygotes to carry out postfertilization partitioning events, we examined the distributions of germ line-specific P granules. In wild-type embryos, P granules become localized at the posterior end of the one-cell embryo during pronuclear migration (Fig. 3b) and are subsequently passed to the posterior daughter cell (P1) during the first division (Strome and Wood, 1983). During the next few divisions, P granules are partitioned to P2, to P3, and finally to the germ line progenitor cell, P4, and its daughters, Z2 and Z3 (Strome and Wood, 1982).

In the embryos produced by older spe-11(hc77ts) hermaphrodites at restrictive temperature, P granules appear to be segregated correctly during the first few cell cycles, regardless of spindle orientation. That is, P granules are seen at the cortex of the embryo opposite the polar body(s) (Fig. 3d). This suggests that P granules are segregated correctly in these embryos. In older hc77ts embryos, P granules are usually concentrated in a subset of cells in the embryo, but are often seen in more than the usual number of two cells. In spe-11(hc90) embryos, P granules appear to be localized to a discrete region of the cytoplasm (Figs. 3e, 3f), and in some embryos they are clearly localized in the cortex (Fig. 3f). However, since it is not possible to determine the anterior–posterior axis or the orientation of these embryos, it is not clear whether the granules are segregated to the “posterior” cortex of the embryo.

Morphological Defects in the Sperm from spe-11 Males

Sperm produced by homozygous spe-11(hc90) and (hc77ts) worms appear normal at the light microscopic level and are motile when activated (L'Hernault et al., 1988). To identify sperm defects that might explain the defects in embryo development, we examined sperm from mutant males by electron microscopy. In C. elegans, the formation of haploid spermatids marks the stage at which the cells become transcriptionally and translationally inactive, the spermatid nucleus is highly condensed, and the spermatocyte ribosomes are left behind in a residual body during the final stage of meiosis (for a recent review see Kimble and Ward, 1988). Among the changes that immediately follow the separation of the spermatids from the residual body are the formation of an electron-dense perinuclear halo and the maturation of a sperm-specific organelle known as the membranous organelle (Ward et al., 1981).

Spermatids from spe-11(hc90 or hc90/sDf4) males differ from wild-type spermatids in their electron-dense perinuclear halo. In wild-type spermatids, the halo completely surrounds the nucleus and is of uniform thickness, except for a slight bulge where it encircles the centriole (Figs. 5a, 5b). In spermatids from spe-11 males there is an excessive amount of perinuclear material around the centriole (Figs. 5a, 5b). In spermatids from spe-11 males there is an excessive amount of perinuclear material around the centriole and only small patches around the rest of the nucleus (Fig. 5c). The material around the centriole is aberrant not only in distribution but also in appearance; it includes many extremely electron-dense, small, roughly spherical particles. These defects are identical in homozygous hc90 and hemizygous hc90/sDf4 animals (>50 spermatids scored). The centriole itself is present and appears normal.
In contrast to sperm from hc90 worms, no perinuclear defects are detectable in sperm from hc77ts animals grown at restrictive temperature; in fact, the mutant spermatids cannot be distinguished morphologically from wild-type. Therefore, the variability in embryonic defects caused by sperm from spe-11(hc77) worms cannot be correlated with variability in perinuclear defects. Sperm from spe-11(hc90) and spe-11(hc77) worms do not have any other visible defects. Thus they are not generally defective in spermatid maturation.

Since one of the components that the sperm contributes to the newly formed zygote in C. elegans is the centrosome and the only defect observed by electron microscopic analysis of mutant sperm is a variation in the appearance of the pericentriolar material, it is possible that the spe-11 defect may be in the microtubule-organizing centers of the early embryo. To test this idea, we stained early spe-11 embryos with anti-centrosome and anti-tubulin antibodies. By immunofluorescence microscopy, spe-11(hc90) and (hc77ts) embryos contain normal appearing centrosomal spindle poles and microtubule arrays (Figs. 6a, 6b), although as expected from the Nomarski phenotype, older spe-11 embryos often contain multiple spindles per cell (Figs. 6a, 6b). Thus, the microtubule-organizing centers contributed to embryos by sperm from spe-11 worms do not show any gross defects in appearance or distribution of microtubules in spindle arrays.

**DISCUSSION**

Unlike other spermatogenesis-defective mutations in C. elegans (L'Hernault et al., 1988), mutations in the spe-11 gene do not affect the production or fertilization-competence of sperm, but rather affect the sperm's ability to participate in initiating normal embryogenesis. The finding that mating homozygous spe-11 hermaphrodites with wild-type males restores the hermaphrodites' ability to produce viable embryos indicates that the spe-11 defect is sperm-specific (L'Hernault et al., 1988). This specificity has been further shown by demonstrating that fertilization of wild-type oocytes with sperm from homozygous mutant males leads to the same embryonic defects observed when mutant oocytes are fertilized by mutant sperm in homozygous spe-11 hermaphrodites. Thus, wild-type spe-11 function cannot be supplied maternally.
The spe-11 gene is probably expressed in spermatocytes during spermatogenesis, since both genotypes of sperm produced by heterozygous spe-11/+ males are capable of producing viable embryos. However, an alternative possibility is that the spe-11 gene product is synthesized by somatic cells and incorporated or imported into mature sperm. Clarification of this point requires either mosaic analysis or cloning of the gene and localization of the gene product. In either case, when spermatids from homozygous hc77ts animals are shifted to restrictive temperature, they express the mutant phenotype. This finding indicates that the temperature-sensitive gene product is present in spermatids and that the gene product’s function can be inactivated after sperm transfer to hermaphrodites. Whether the gene product is stored in spermatids as RNA or protein remains to be determined.

The lack of a dominant effect of the spe-11 mutation suggests that mutant spe-11 product does not poison embryos, but rather that spe-11+ product is needed for normal development. The argument that a spe-11/+ heterozygote simply does not produce enough poison to lead to embryonic lethality is refuted by the normal functioning of sperm from spe-11(hc90)+ and spe-11(hc90)/+ worms (two mutant allele-bearing chromosomes plus a free duplication carrying a wild-type copy of the gene). In addition, sperm from hemizygous spe-11(hc90)/SDF4 hermaphrodites produce abnormal embryos with the same phenotype as embryos produced by hc90 homozygotes; this result suggests that the hc90 allele is either a null allele or a severe hypomorph. The results of L’Hernault et al. (1988) strengthen the likelihood that hc90 is a severe hypomorph; hermaphrodites carrying hc77ts in trans to hc90 produce a few more viable progeny than hemizygous hc77ts/SDF4 hermaphrodites.

The earliest defects observed in spe-11 embryos are what may be considered activation defects: failure to complete meiosis and failure to produce a rigid, impermeable, oval eggshell. Because of the latter defect, spe-11(hc90) and hc77ts embryos are more spherical than normal, misshapen, and osmotically sensitive. Yet, despite their poor eggshell, spe-11 embryos do not appear to be defective in blocking polyspermy. Although the early defects may be activation defects, it is clear that the spe-11 mutation does not result in a complete failure to activate the egg, since many of the events that occur after fertilization by wild-type sperm also occur in eggs fertilized by sperm from spe-11 mutants. spe-11 ++
embryos appear to undergo pseudocleavage contractions although the contractions are not ordered as they are in normal embryos. This may be due to the lack of a rigid eggshell for the embryo to “push” against as the cytoplasm streams and the cortex contracts or it may reflect an altered egg cortex resulting from fertilization by a mutant sperm. Pronuclear migration in spe-11 embryos appears normal, with one pronucleus migrating the length of the embryo to meet the other, and then both migrating to the center of the embryo, where the mitotic spindle is established. In spe-11(hc77ts) embryos, the segregation of P granules appears to occur correctly; they become localized at the cortex of the one-cell embryo opposite the polar bodies. This result suggests that these embryos do possess an anterior–posterior axis. In spe-11(hc90) embryos, P granules appear to be segregated to a restricted region along the cortex, but since there are no polar bodies to serve as anterior markers, it is not possible to determine whether or not this region represents the “posterior” cortex of the embryo.

In addition to “activation defects,” spe-11 embryos are defective in orienting their mitotic spindles and in completing cytokinesis. In wild-type embryos, the first mitotic spindle is initially oriented perpendicularly to the anterior–posterior axis, but rotates to become oriented along the anterior–posterior axis. Using the path of pronuclear migration as the only indicator of the anterior–posterior axis, the mitotic spindle in spe-11(hc90 and hc77ts) embryos does not rotate and often remains oriented perpendicularly to the path of pronuclear migration. This failure is probably not due to the altered shape of the embryos, since wild-type embryos that are rendered spherical by chitinase digestion of their eggshell undergo normal spindle rotation (Hyman and White, 1987). Nor is it likely to be due to the failure of the embryos to complete meiosis, since embryos whose meiotic divisions are inhibited by transient treatment with microfilament inhibitor progress through early embryogenesis apparently normally (Hill and Strome, unpublished). Despite what appears to be a normal progression through the stages of mitosis, spe-11(hc90) embryos usually fail to complete cytokinesis and therefore remain unicellular. Since the position of the mitotic spindle is ultimately responsible for the site of cleavage furrow formation, this defect may be a result of altered spindle placement. spe-11(hc77ts) embryos usually do complete cytokinesis. They continue to divide to a few hundred cells but do not show any signs of morphogenesis. Also, due to the defects in spindle orientation, the cells generated probably do not contain the normal complements of cytoplasmic components.

The nature of the spe-11 defect remains a mystery. The failure of spe-11 embryos to complete meiosis and form a good eggshell suggests a partial failure in activation. However, the later defects in spindle orientation and cytokinesis are not easily explained by aberrant activation. Several possibilities are presented below: (1) Sperm from spe-11 animals may be defective in triggering a physiological change in the oocyte, for example, a change in membrane potential or intracellular ion levels. Such a defect would be expected to affect many processes. (2) Fertilization by sperm from spe-11 worms may result in defects in the egg cortex and/or membrane. Such defects could impair secretion or formation of the eggshell, prevent normal interactions between the mitotic spindle and the cortex, and impair cleavage furrow formation. (3) Sperm from spe-11 worms may contribute defective microtubule-organizing centers. The finding that oocytes of some species are activated by injected centrosomes (centrioles and pericentriolar material) (Picard et al., 1987) suggests that microtubule-organizing centers may provide essential activation functions, in addition to nucleating microtubule arrays. Electron microscopic analysis of sperm from spe-11(hc90) males suggests a defect in microtubule-organizing centers; the electron-dense material surrounding the sperm nucleus and centriole is aberrantly distributed and unusual looking. However, sperm from hc77ts males contain normal appearing perinuclear halos and yet result in abnormal embryogenesis, suggesting that the halo defect seen in sperm from hc90 worms is not the cause of the subsequent embryonic defects. Interestingly, three other spermatogenesis-defective mutants, fer-2, fer-3, and fer-4, also have a defective perinuclear halo, although the defective halos contain “tubules” rather than granules. Sperm from these mutants are for the most part fertilization-defective, although they do occasionally produce inviable embryos (Ward et al., 1981).

Whatever the nature of the spe-11 gene product, based on the paternal-effect embryonic-lethal phenotype of the alleles described, its function appears to be required only for the correct initiation of embryogenesis. Since homozygous mutant animals (produced by spe-11/+ hermaphrodites or by mating spe-11 hermaphrodites and spe-11/+ males) grow into healthy adults containing fertile oocytes, the spe-11 gene product is either not utilized at other times in development or can be compensated for by other genes.

Several C. elegans mutations, identified in earlier screens for temperature-sensitive maternal-effect lethal mutants, also display paternal effects. On the basis of the parental effect tests done by Wood et al. (1980), zyg-8 is “paternal”; sperm-supplied wild-type zyg-8 product is sufficient for survival of eggs produced by homozygous mutant worms. Unfortunately, zyg-8 males raised at restrictive temperature fail to transfer
sperm during mating, making it impossible to analyze the development of wild-type oocytes fertilized by mutant sperm. Zygotes produced by homozygous zyg-8 hermaphrodites often display more than two pronuclei and divide into 3 or 4 cells, suggesting entry by more than one sperm. According to parental effect tests, emb-27 and emb-30 are both strict maternal-effect mutants (i.e., maternal expression is necessary) (Isnenghi et al., 1983); yet, maternal expression is not sufficient, since wild-type oocytes fertilized by sperm from mutant males arrest after the 100-cell stage (R. Cassada, personal communication). Two paternal-effect mutations have been identified in Drosophila, pal (for paternal loss) and ms(3)K81. The pal mutation results in defects in male meiosis and chromosome segregation during early embryonic mitoses (Baker, 1975). The ms(3)K81 mutation results in the production of sperm that are defective in syngamy but are able to activate eggs to develop gynogenetically (Fuyama, 1986). The spe-11 gene, which appears to participate, directly or indirectly, in the early ontogeny of C. elegans embryos, differs significantly from the two Drosophila genes, both of which appear to affect primarily chromosome behavior.

The existence of the spe-11 mutations demonstrates that sperm functions or factors are essential for execution of the maternal instructions that are generally thought to guide early C. elegans embryogenesis. Systematic screens for more paternal-effect embryonic-lethal mutants are in progress in both C. elegans and Drosophila. Identification and analysis of the roles of the gene products encoded by paternal-effect genes will provide important information about the process of fertilization and the role of sperm factors in the initiation of embryogenesis.

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