The C-terminus of SPE-11 is required for proper embryonic development in C. elegans

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Abstract

SPE-11 is a sperm protein required to initiate embryonic development. Mutations of the spe-11 gene result in a strict paternal-effect lethal phenotype. Wild-type oocytes fertilized by sperm from homozygous spe-11 males leads to abnormal development, but the reciprocal interaction, spe-11 mutant oocytes fertilized by wild-type male sperm, results in normal development. The C-terminus of SPE-11 is required for proper embryonic development in C. elegans.

Figure 1: C-terminal mutations of SPE-11 fail to rescue a spe-11 deletion mutant. A. Cartoon of SPE-11 constructs for in vivo localization studies and functional analyses. All constructs were expressed as GFP fusions from the spe-11 promoter and 3’UTR. Putative nuclear localization signals (NLS) are indicated by vertical black bars. Mutated NLSs are indicated by vertical yellow bars. A yellow star for the GFP::SPE-11AAA mutant indicates the following amino acid changes: K289A, R292A, and K293A. B. Transgenic males expressing different GFP::SPE-11 (green) fusion constructs and mCherry-histone (red) were analyzed for proper localization in the spe-11(ok2143) mutant in diplotene nuclei and post-meiotic sperm. Insets are enlargements of post-meiotic sperm. Scale bar, 5 µm. C. The number of viable progeny produced by spe-11(ok2143) and spe-11(ok2143) expressing gfp::spe-11, gfp::spe-11NLS1mut, gfp::spe-11AAAmut, gfp::spe-11NLS5mut, or gfp::spe-11Δ295-299 (Error bars, SD. **p<0.001, *p<0.05).

Description

SPE-11 is a sperm protein required to initiate embryonic development. Mutations of the spe-11 gene result in a strict paternal-effect lethal phenotype. Wild-type oocytes fertilized by sperm from homozygous spe-11 males leads to abnormal development, but the reciprocal interaction, spe-11 mutant oocytes fertilized by wild-type male sperm, results in normal development.
SPE-11 is a 299 amino acid protein with no significant similarity to other proteins. SPE-11 is first detected in spermatoocytes in the gonad of males as foci that eventually unite to form a ring around the condensed DNA of mature sperm (Browning and Strome 1996). It is predicted to be a very highly hydrophilic protein with five potential nuclear localization sequences (NLS) (Browning and Strome 1996). SPE-11 has no obvious protein domains (Browning and Strome 1996). To define functional units of the SPE-11 protein, we generated a series of GFP::SPE-11 mutant constructs along with a GFP::SPE-11 rescuing construct (Figure 1A) and created transgenic worm lines in the null spe-11(ok2143) background (deletion of exons two through six out of seven total exons) through microparticle bombardment. To determine functionality of each SPE-11 mutant construct we assayed the transgenic lines for SPE-11 localization in late meiotic prophase and in post-meiotic sperm and for rescue of the embryonic lethality phenotype. Similar to previous reports of SPE-11 localization, the wild-type GFP::SPE-11 construct forms foci around diplotene nuclei that eventually coalesce into a perinuclear ring in spermatids (Browning and Strome 1996) and rescues embryonic lethality (Avg. number of viable progeny=175.3, Figure 1B and 1C).

We started our mutant analysis by creating a single integrated, transgenic line carrying a mutant version of the first putative NLS (GFP::SPE-11NLS1). GFP::SPE-11NLS1 both rescues the embryonic lethality of the spe-11(ok2143) mutant and localizes correctly in the male germ line (Figure 1B and 1C). We have also generated a single integrated line with the fifth putative NLS mutated (GFP::SPE-11NLS5). This line localizes correctly during the diplotene stage of meiotic prophase, however it fails to encircle the nuclei of post-meiotic sperm and fails to rescue the embryonic lethality of the spe-11(ok2143) mutant (Figure 1B and 1C). This failed rescue along with the localization defect suggests that the amino acids mutated in GFP::SPE-11NLS5 are important for SPE-11 function and localization. We also generated a SPE-11 construct in which three basic amino acids in the last ten amino acids of the C-terminus are changed to Ala (GFP::SPE-11AAAmut). Note that the GFP::SPE-11NLS5 mutant has two additional basic residues changed to Ala (see Reagents section). Interestingly, the GFP::SPE-11AAAmut mutant had a wild-type localization pattern, yet it completely fails to rescue the embryonic lethality of the spe-11(ok2143) mutant (Avg. number of viable progeny=4.6, Figure 1B and 1C).

The majority of existing spe-11 mutant alleles truncate the SPE-11 protein suggesting that the C-terminus is very important for function. To test the importance of the C-terminus on SPE-11 function we generated an additional transgenic line, one in which only the last five amino acids are removed (GFP::SPE-11Δ295-299). This line has a significantly lower number of viable progeny than the GFP::SPE-11 rescuing construct (p<0.05, Figure 1B). GFP::SPE-11Δ295-299 localization was similar to GFP::SPE-11 in diplotene stage nuclei, however, it fails to localize correctly in post-meiotic sperm (Figure 1C) suggesting that even the most C-terminal end of the protein (i.e. the last five amino acids) is essential for localization. Interestingly, while embryonic viability was severely reduced in the GFP::SPE-11Δ295-299 line (Avg. number of viable progeny=31.9, Figure 1B and 1C), several progeny survived to adulthood.

In summary, we have identified several critical amino acids required for proper SPE-11 localization and function. The putative NLS5 and the extreme C-terminus are required for both localization and function. While not required for localization, analysis of the GFP::SPE-11AAA mutant has identified three critical amino acids in the C-terminus that are required for SPE-11 function. Recreation of these alleles in the endogenous spe-11 gene, using CRISPR/Cas9 genome editing, would be interesting to pursue.

Methods
Request a detailed protocol

All strains were cultured using standard conditions (Brenner 1973). For the creation of transgenic spe-11 animals, the promoter region, open reading frame, and 3’UTR of spe-11 were cloned into separate entry vectors using Gateway technology (Invitrogen). These three entry clones plus an entry clone carrying GFP were recombined into a destination vector with a wild-type unc-119 gene. Mutant unc-119(ed3) animals were transformed by biolistic transformation (or bombardment) with the destination clone (Wilm et al. 1999). Postbombardment, non-Unc animals were picked to determine which lines were integrated and which were extrachromosomal lines. At least two lines were generated for each spe-11 construct and only integrated lines were used for analysis in this study. Lines carrying the spe-11 transgenes were
crossed into the spe-11(ok2143) background balanced over hT2. To visualize chromosomes, itIs37 [mCherry::H2B] was crossed into each spe-11(ok2143)/hT2 transgenic line. All analysis was performed on animals homozygous for spe-11(ok2143), itIs37 [mCherry::H2B], and the spe-11 transgene. The average number of live progeny for each strain was determined by picking single L4 hermaphrodites and transferring the animal to fresh plates every 24 h until no additional embryos were produced. The number of hatched larvae were counted after each 24-hour period. SPE-11 localization was determined through live image analysis of adult males. Adult males 24 h post L4 were anesthetized with 2 mM levamisole (Sigma), mounted on an agarose pad, and covered with a coverslip. Image collection was performed using a Nikon Eclipse E800 spinning disk confocal microscope and MetaMorph imaging software. Images were processed and analyzed using Fiji (Schindelin et al. 2012).

Reagents
AG583: spe-11(ok2143)/hT2 I; itIs37 [pie-1p::mCherry::his-58 + unc-119(+)]
AG584: spe-11(ok2143)/hT2 I; avIs146 [pUNC-119(+) + spe-11p::GFP::spe-11::spe-11 3’UTR]; itIs37
AG585: spe-11(ok2143)/hT2 I; avIs149 [SPE-11 NLS1 mutant: PKKKS (amino acids 18-22) to PAAAS]; itIs37
AG586: spe-11(ok2143)/hT2 I; avIs152 [SPE-11A295-299]; itIs37
AG587: spe-11(ok2143)/hT2 I; avIs159 [SPE-11AAA mutant; Sequence change: KFYRK (amino acids 289-293) to AFYAA]; itIs37
AG588: spe-11(ok2143)/hT2 I; avIs161 [SPE-11NLS5 mutant; Sequence change: KKSLSVVANVQAFYAA (amino acids 277-293) to KKSLSAVANVQAFYAA]; itIs37

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