Caenorhabditis elegans PIEZO Channel Coordinates Multiple Reproductive Tissues to Govern Ovulation

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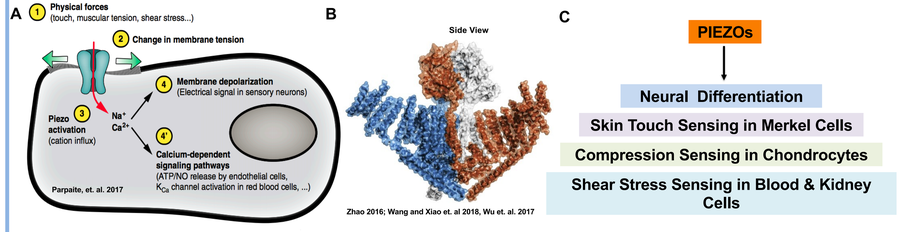
Abstract

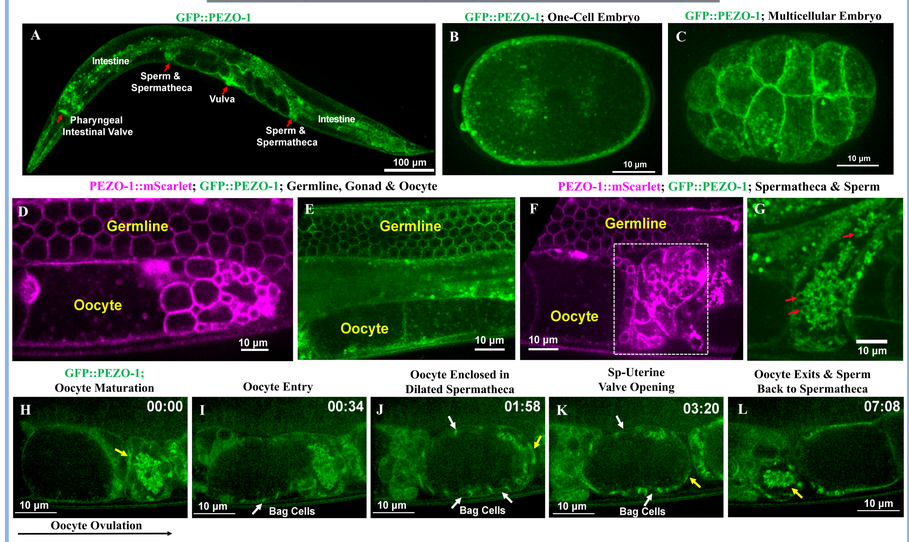
The PIEZO proteins are involved in a wide range of developmental and physiological processes. Human PIEZO1 and PIEZO2 are newly identified excitatory mechano-sensitive proteins; they are non-selective ion channels that exhibit a preference for calcium in response to mechanical stimuli. To further understand the function of these proteins, we investigated the roles of *pezo-1*, the sole *PIEZO* ortholog in *C. elegans*. *pezo-1* is expressed throughout development in *C. elegans*, with strong expression in reproductive tissues. A number of deletion alleles as well as a putative gain-of-function mutant caused severe defects in reproduction. A reduced brood size was observed in the strains depleted of PEZO-1. *In vivo* observations show that oocytes undergo a variety of transit defects as they enter and exit the spermatheca during ovulation. Post ovulation oocytes were frequently damaged during spermathecal contraction. Calcium signaling in the spermatheca is normal during ovulation in *pezo-1* mutants, however, *pezo-1* interacts genetically with known regulators of calcium signaling. Lastly, loss of PEZO-1 caused defective sperm navigation after being pushed out of the spermatheca during ovulation. Mating with males rescued these reproductive deficiencies in our *pezo-1* mutants. These findings suggest that PEZO-1 may act in different reproductive tissues to promote proper ovulation and fertilization in *C. elegans*.

PIEZO Proteins: Mechanically Activated Ion Channels



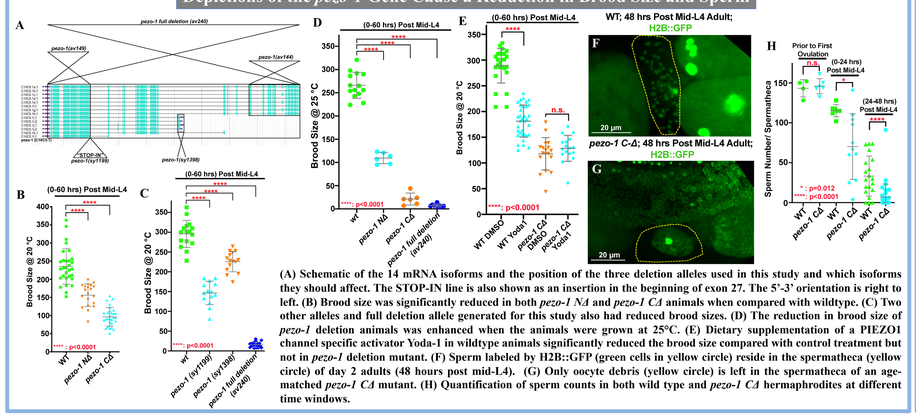
Piezo is a novel ion channel protein which is involved in cell mechanotransduction to convert mechanical forces into biological signals (A). PIEZO proteins contain over 2500 amino acids with 26-40 transmembrane segments. Cryo-EM structure of Piezo shows a three-bladed, propeller-shaped trimeric complex, including ion-conducting pore module at C-terminal region, and transmembrane segments at N-terminal region (B). In human, there are two PIEZOs proteins identified, PIEZO1 and PIEZO2. Both proteins express widely in human body and are involved in multiple physiological processes, such as shear-stress sensing, neural differentiation (C).

PEZO-1 Expresses in Multiple Reproductive Tissues in *C. elegans*



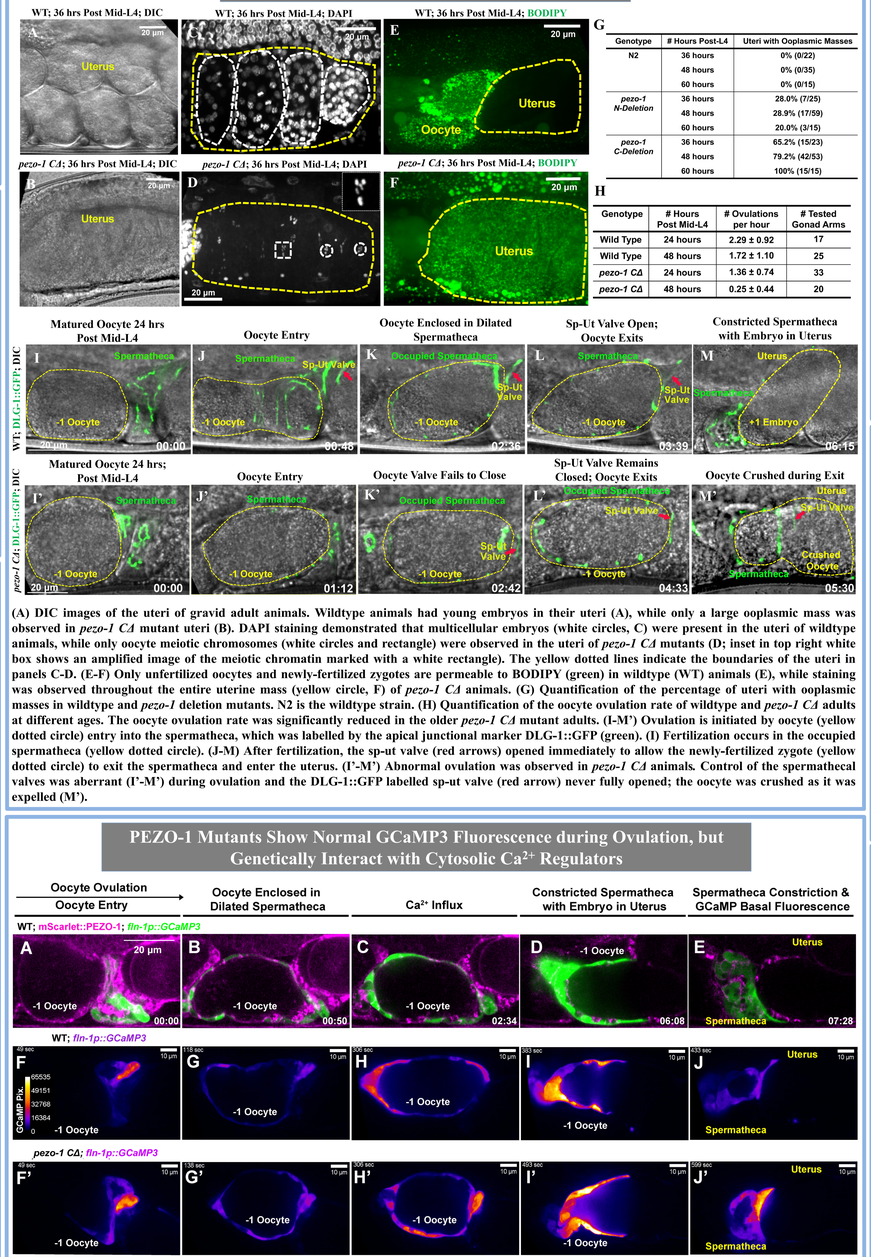
(A) GFP::PEZO-1 is strongly expressed in multiple mechanosensitive tissues, such as the pharyngeal-intestinal valve, spermatheca, and vulva (red arrows). (B-C) GFP::PEZO-1 (green) is expressed in the plasma membrane of different staged embryos. (D-G) Both PEZO-1::mScarlet (magenta) and GFP::PEZO-1 (green) localize to reproductive tissues, such as the plasma membrane of the germline cells (D-E), somatic gonad (D-F), spermatheca (F; in white box), and sperm (red arrows) (G). (H-L) Representative images of PEZO-1 localization during ovulation and fertilization. GFP::PEZO-1 (green) localizes to the spermathecal distal valve (yellow arrow, H), which remains closed before ovulation. The oocyte ovulated and entered into the spermatheca (I) and stayed enclosed in the spermatheca until fertilization completed (J). During fertilization, GFP::PEZO-1 remained on the spermathecal-uterine (sp-ut) valve as indicated by a yellow arrow (J, K). The bag cells of the spermatheca also express GFP::PEZO-1 at this time (representative bag cells are marked by white arrows, I-K). After fertilization, the sp-ut valve opened (K, yellow arrow) and allowed the newly-fertilized zygote to exit the constricting spermatheca (L). Once a fertilized zygote entered the uterus, the spermatheca constricts; sperm can be seen in the constricted spermatheca (L, yellow arrow). Black arrow below panel J shows the direction the embryo travels through the spermatheca from left to right. Timing of each step is labeled on the top right in minutes and seconds. Scale bars are indicated in each panel.

Depletions of the *pezo-1* Gene Cause a Reduction in Brood Size and Sperm



(A) Schematic of the 14 mRNA isoforms and the position of the three deletion alleles used in this study and which isoforms they should affect. The STOP-IN line is also shown as an insertion in the beginning of exon 27. The 5'-3' orientation is right to left. (B) Brood size was significantly reduced in both *pezo-1 NΔ* and *pezo-1 CΔ* animals when compared with wildtype. (C) Two other alleles and full deletion allele generated for this study also had reduced brood sizes. (D) The reduction in brood size of *pezo-1* deletion animals was enhanced when the animals were grown at 25°C. (E) Dietary supplementation of a PIEZO1 channel specific activator Yoda-1 in wildtype animals significantly reduced the brood size compared with control treatment but not in *pezo-1* deletion mutant. (F) Sperm labeled by H2B::GFP (green cells in yellow circle) reside in the spermatheca (yellow circle) of day 2 adults (48 hours post mid-L4). (G) Only oocyte debris (yellow circle) is left in the spermatheca of an age-matched *pezo-1 CΔ* mutant. (H) Quantification of sperm counts in both wild type and *pezo-1 CΔ* hermaphrodites at different time windows.

PEZO-1 Mutants Cause Severe Ovulation Defects



Genotype	# Hours Post-L4	Uteri with Ooplasmic Masses
N2	36 hours	0% (0/22)
	48 hours	0% (0/35)
	60 hours	0% (0/15)
pezo-1 N-Deletion	36 hours	28.0% (7/25)
	48 hours	28.9% (17/59)
	60 hours	20.0% (3/15)
pezo-1 C-Deletion	36 hours	65.2% (15/23)
	48 hours	79.2% (42/53)
	60 hours	100% (15/15)

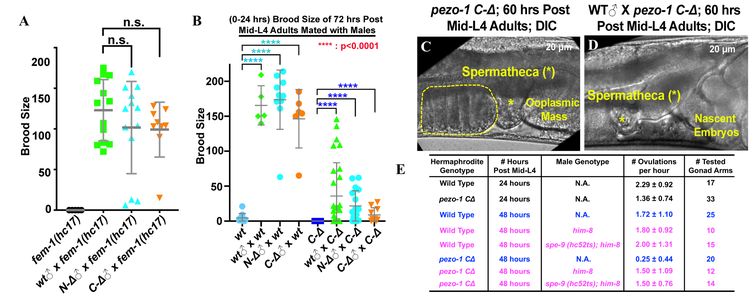
Genotype	# Hours Post Mid-L4	# Ovulations per hour	# Tested Gonad Arms
Wild Type	24 hours	2.29 ± 0.92	17
Wild Type	48 hours	1.72 ± 1.10	25
pezo-1 CΔ	24 hours	1.36 ± 0.74	33
pezo-1 CΔ	48 hours	0.25 ± 0.44	20

(A) DIC images of the uteri of gravid adult animals. Wildtype animals had young embryos in their uteri (A), while only a large ooplasmic mass was observed in *pezo-1 CΔ* mutant uteri (B). DAPI staining demonstrated that multicellular embryos (white circles, C) were present in the uteri of wildtype animals, while only oocyte meiotic chromosomes (white circles and rectangle) were observed in the uteri of *pezo-1 CΔ* mutants (D; inset in top right white box shows an amplified image of the meiotic chromatin marked with a white rectangle). The yellow dotted lines indicate the boundaries of the uteri in panels C-D. (E-F) Only unfertilized oocytes and newly-fertilized zygotes are permeable to BODIPY (green) in wildtype (WT) animals (E), while staining was observed throughout the entire uterine mass (yellow circle, F) of *pezo-1 CΔ* animals. (G) Quantification of the percentage of uteri with ooplasmic masses in wildtype and *pezo-1* deletion mutants. N2 is the wildtype strain. (H) Quantification of the oocyte ovulation rate of wildtype and *pezo-1 CΔ* adults at different ages. The oocyte ovulation rate was significantly reduced in the older *pezo-1 CΔ* mutant adults. (I-M') Ovulation is initiated by oocyte (yellow dotted circle) entry into the spermatheca, which was labelled by the apical junctional marker DLG-1::GFP (green). (I) Fertilization occurs in the occupied spermatheca (yellow dotted circle). (J-M) After fertilization, the sp-ut valve (red arrows) opened immediately to allow the newly-fertilized zygote (yellow dotted circle) to exit the spermatheca and enter the uterus. (I'-M') Abnormal ovulation was observed in *pezo-1 CΔ* animals. Control of the spermathecal valves was aberrant (I'-M') during ovulation and the DLG-1::GFP labelled sp-ut valve (red arrow) never fully opened; the oocyte was crushed as it was expelled (M').

PEZO-1 Mutants Show Normal GCaMP3 Fluorescence during Ovulation, but Genetically Interact with Cytosolic Ca^{2+} Regulators

(A-E) mScarlet::PEZO-1 colocalizes with GCaMP3 that is driven by a spermatheca-specific promoter. These images represent the third ovulation for this animal. (F-J') Time series frames from GCaMP3 recordings in the third ovulation of both wildtype animals (F-J) and *pezo-1 CΔ* animals (F'-J'). Ca^{2+} influx was quantified during ovulation and fertilization, as indicated by the intensity of GCaMP3 pixels (colorized bar in F). (F, F') Oocyte entry into the spermatheca in wild type and *pezo-1 CΔ*. (G, G') Oocytes in the spermatheca, (H, H') Ca^{2+} influx during fertilization, (I, I') intense Ca^{2+} influx as sp-ut valve closes to push newly-fertilized zygote into the uterus, and (J, J') the return to basal levels as the spermatheca prepares for the next ovulation. (K) Dwell time is a tissue function metric calculated as the time the oocyte resides in the spermatheca from the closing of the distal valve to the opening of the sp-ut valve. Black arrow above panel A shows the direction the embryo travels through the spermatheca from left to right. Timing of each step is labeled on the bottom right in minutes and seconds (A-E). (L-N) *itr-1 (RNAi)* reduced the brood size in *pezo-1 CΔ* animals. (M) In contrast, *lfe-2 (RNAi)* slightly rescued the smaller brood size in *pezo-1 CΔ* animals. (N) Depletion of both *orai-1* and *sca-1* by RNAi also enhanced the brood size reduction of *pezo-1 CΔ* mutants. P-values: * = 0.025 (C); ** = 0.0048 (A); * = 0.0001 (B); **** <0.0001 (*t*-test). Scale bars are indicated in each panel.**

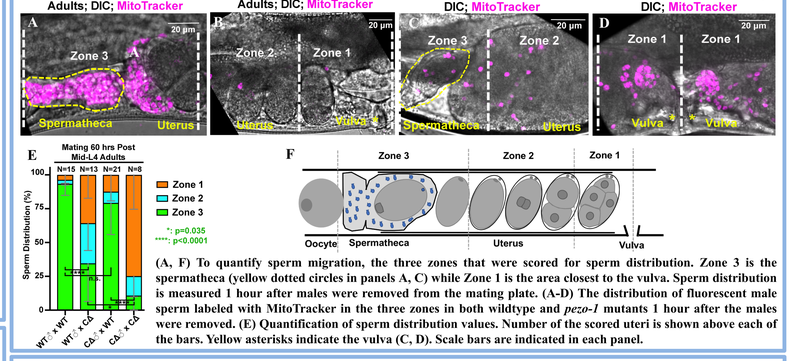
Male Sperm Rescue the Ovulation Defects in *pezo-1* Mutants



Hermaphrodite Genotype	# Hours Post Mid-L4	Male Genotype	# Ovulations per hour	# Tested Gonad Arms
Wild Type	24 hours	N.A.	2.29 ± 0.92	17
pezo-1 CΔ	24 hours	N.A.	1.36 ± 0.74	33
Wild Type	48 hours	N.A.	1.72 ± 1.10	25
Wild Type	48 hours	*him-8*	1.80 ± 0.92	10
Wild Type	48 hours	*spe-9 (hc52ts); him-8*	2.00 ± 1.31	15
pezo-1 CΔ	48 hours	N.A.	0.25 ± 0.44	20
pezo-1 CΔ	48 hours	*him-8*	1.50 ± 1.09	12
pezo-1 CΔ	48 hours	*spe-9 (hc52ts); him-8*	1.50 ± 0.76	14

(A) Both *pezo-1 CΔ* and *NΔ* males are fertile and sire progeny when mated with *fem-1(hc17ts)* mutants (essentially female animals). (B) Mating with male sperm rescued fertility in day 3 *pezo-1 CΔ* adults (72 hours post mid-L4). (C) The oocyte maturation and ovulation rate are very low in day 3 *pezo-1 CΔ* mutant adults and oocytes accumulate in the proximal gonad arm (yellow dashed circle). (D) In contrast, the ovulation rates are recovered to high levels after mating with wildtype male sperm. Newly-fertilized embryos pushed the ooplasmic mass out of the uterus. Yellow asterisk indicates the spermatheca (C, D). (E) Quantification of the oocyte ovulation rate of wildtype and *pezo-1 CΔ* adults at different ages. *spe-9 (hc52ts)* sperm significantly rescue ovulation rates in *pezo-1 CΔ* hermaphrodites even though they do not fertilize oocytes. P-values: ** <0.0001 (*t*-test). Scale bars are indicated in each panel.**

Sperm Guidance and Navigation is Disrupted in *pezo-1* Mutants



(A, F) To quantify sperm migration, the three zones that were scored for sperm distribution. Zone 3 is the spermatheca (yellow dotted circles in panels A, C) while Zone 1 is the area closest to the vulva. Sperm distribution is measured 1 hour after males were removed from the mating plate. (A-D) The distribution of fluorescent male sperm labeled with MitoTracker in the three zones in both wildtype and *pezo-1* mutants 1 hour after the males were removed. (E) Quantification of sperm distribution values. Number of the scored uteri is shown above each of the bars. Yellow asterisks indicate the vulva (C, D). Scale bars are indicated in each panel.

Tissue-Specific Degradation of PEZO-1 Displays a Reduced Brood Size and Causes Sperm Navigational Defects

(A) A degron tag was inserted at the 3' end of the *pezo-1* coding sequence using CRISPR/Cas9-mediated editing. (B) Brood size was reduced in all degron strains when animals were treated with 2 mM auxin. Data are presented as the mean ± standard error from at least two independent experiments. (C-F) Sperm distribution 1 hour after male removal from mating plates. The germline specific PEZO-1::Degron hermaphrodites were mated with wildtype males for 30 minutes. The representative images show that *pezo-1* degradation in the germ line influences sperm distribution from the vulva (zone 1) to the spermatheca (zone 3). (G) Quantification of sperm distribution in the PEZO-1::Degron strain grown on plates with (+) or without (-) 2 mM auxin. Scale bars are indicated in each panel.

A *PIEZO1* Disease Allele Causes Severe Brood Size Reduction in *C. elegans*

Genotype	# of hrs Post Mid-L4	Uteri with Ooplasmic Mass
pezo-1 (R2405P)	36 hours	100% (22/22)
	60 hours	100% (22/22)

Genotype	# Hours Post Mid-L4	Male Genotype	# Ovulations per hour	# Tested Gonad Arms
pezo-1 (R2405P)	24 hours	N.A.	2.17 ± 1.34	12
pezo-1 (R2405P)	48 hours	N.A.	0.10 ± 0.32	10
pezo-1 (R2405P)	48 hours	*spe-9 (hc52ts); him-8*	1.53 ± 0.92	16

(A) Sequence alignment showing arginine 2405 (R2405) in *C. elegans* PEZO-1 is highly conserved with human and mouse PIEZO1 and PIEZO2. (B) A conserved patient-specific allele *pezo-1(R2405P)* was generated and causes uterine ooplasmic masses (B) and a severe reduction in brood size (C). (D) *itr-1 (RNAi)* enhanced the brood size reduction of *pezo-1 (R2405P)* mutants, while *lfe-2 (RNAi)* slightly rescued the reduced brood size. (E) *spe-9 (hc52ts)* sperm rescued the very low ovulation rate in *pezo-1 (R2405P)* hermaphrodites.

Conclusion

1) *pezo-1* expresses at multiple reproductive tissues, including spermatheca, oocytes and sperm.
2) Depletion of *pezo-1* causes severe reproductive deficiency, including reduced brood size and sperm.
3) *pezo-1* mutant genetically interacts with cytosolic Ca^{2+} regulators.
4) Male sperm rescue ovulation defects in *pezo-1* mutants.
5) Tissue-specific degradation of PEZO-1 caused reduced brood size and sperm navigation defects.
6) A patient-specific allele causes severe brood size reduction and ovulation defects.

Future suppressor screens with this and other *pezo-1* patient-specific alleles should help identify other genetic interactors.