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Insulin-like signalling to the maternal germline controls progeny response to osmotic stress

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Abstract

In 1893 August Weismann proposed that information about the environment could not pass from somatic cells to germ cells[1], a hypothesis now known as the Weismann barrier. However, recent studies have indicated that parental exposure to environmental stress can modify progeny physiology[2–7] and that parental stress can contribute to progeny disorders[8]. The mechanisms regulating these phenomena are poorly understood. We report that the nematode *C. elegans* can protect itself from osmotic stress by entering a state of arrested development and can protect its progeny from osmotic stress by increasing the expression of the glycerol biosynthetic enzyme GPDH-2 in progeny. Both of these protective mechanisms are regulated by insulin-like signalling: insulin-like signalling to the intestine regulates developmental arrest, while insulin-like signalling to the maternal germline regulates glycerol metabolism in progeny. Thus, there is a heritable link between insulin-like signalling to the maternal germline and progeny metabolism and gene expression. We speculate that analogous modulation of insulin-like signalling to the germline is responsible for effects of the maternal environment on human diseases that involve insulin signalling, such as obesity and type-2 diabetes[8].

Keywords

Heritable; Insulin; MAP kinase; Osmotic stress; Germline

Maternal exposure to a wide variety of environmental stresses alters progeny growth, development, and physiology of diverse organisms[2–7] and is thought to be a contributing factor to several human pathologies, including obesity and diabetes[8]. The mechanisms by

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The authors declare that they have no competing financial interests

which the maternal environment can modify progeny biology are poorly understood. Parental exposure of the nematode *C. elegans* to mild osmotic stress can protect progeny from the effects of strong osmotic stress[9]. This finding and similar observations of other organisms[2] suggest that besides the potentially deleterious effects of maternal environmental stress on progeny, maternal exposure to environmental stress might epigenetically precondition progeny and protect them from similar environmental insults in the future. How maternal exposure to environmental stress can protect progeny from future environmental stress remains largely unknown.

To determine how parental exposure to mild osmotic stress (300 mM NaCl) protects progeny from the effects of strong osmotic stress (500 mM NaCl) we first examined the effects of 500 mM NaCl on *C. elegans*. Embryos placed at 500 mM NaCl completed embryonic development and hatched but arrested development immediately after hatching (Fig. 1a and Supplementary Fig. 1a). These arrested animals were unable to move, feed, or respond to touch (Supplementary Fig. 1a). However, when arrested animals were returned to normal osmotic conditions (50 mM NaCl), they regained mobility and resumed development (Fig. 1b). We made similar observations when NaBr, KCl, or sucrose was used to cause osmotic stress (Supplementary Fig. 1b–d). We conclude that young *C. elegans* larvae can enter a state of immobile arrested development in response to osmotic stress.

C. elegans also arrests development in response to other environmental stresses, such as starvation[10–11]. These arrests are caused by the loss of insulin-like signalling via the insulin receptor DAF-2 and the consequent activation of the FOXO transcription factor DAF-16[10–12]. We tested whether the activation of DAF-16 is required for developmental arrest in response to osmotic stress. Animals lacking DAF-16 were less likely than the wild type to arrest development in response to osmotic stress (Fig. 1a), whereas animals with increased DAF-16 activation, such as *daf-2* mutants[12], were more likely to arrest development in response to osmotic stress (Fig. 1a). Genes in other stress response pathways, such as those that regulate the responses of *C. elegans* to oxidative stress[13] or infection[14], were not required for developmental arrest in response to osmotic stress (Supplementary Fig. 1e–1f). In addition, DAF-2 activity in the intestine was required for development at 300 mM NaCl (Fig. 1c), and exposure to 500 mM NaCl caused the translocation of DAF-16 from the cytoplasm to the nucleus (Fig. 1d). Like the developmental arrest caused by starvation, the arrest caused by osmotic stress was regulated by dense-core vesicle release[10]; furthermore, we found that it is dense-core vesicle release from sensory neurons that regulates developmental arrest in response to osmotic stress (Supplementary Fig. 1g), and *daf-16* mutants, which are resistant to developmental arrest, showed an increased susceptibility to osmotic stress (Fig. 1b). These results indicate that like starvation, osmotic stress causes developmental arrest by inhibiting insulin-like signalling and that arrested development correlates with enhanced survival.

In contrast to starvation induced developmental arrest, we found that developmental arrest in response to osmotic stress is regulated by a different insulin-like peptide, INS-3 (Supplementary Fig. 1h–1i and Supplementary Table 1), than arrest in response to starvation (INS-4 and DAF-28[15]) and that animals that arrest development in response to osmotic stress are immobile and unable to respond to touch, unlike arrest in response to starvation in

which animals remain mobile[10]. In addition, we found that a majority (78%) of genes the expression of which reproducibly changed in response to osmotic stress were not affected by starvation (Supplementary Fig. 2 and Supplementary Table 2). These results suggest that these two arrest phenotypes are controlled by partially overlapping but distinct pathways.

Frazier and Roth (2009) found that parental exposure of *C. elegans* to mild osmotic stress protects progeny from the effects of strong osmotic stress and that this protection required DAF-2 activation[9]. These authors described an apparently anomalous result: *daf-2; daf-16* double mutants were significantly better at adaptation to osmotic stress than both wild-type animals and either *daf-2* or *daf-16* single mutants[9]. We hypothesized that this anomaly might be at least in part caused by differing effects of maternal insulin-like signalling and progeny insulin-like signalling with respect to progeny response to osmotic stress. Specifically, we suspected that DAF-2 activation in embryos was required for adaption to osmotic stress, consistent with both the observations of Frazier and Roth and our findings (Fig. 1a). We also suspected that it was an inhibition rather than an activation of parental insulin-like signalling that resulted in the protection of progeny from osmotic stress, just as the inhibition of larval insulin-like signalling induced by osmotic stress protects larvae from osmotic stress by causing developmental arrest. To test this hypothesis, we crossed wild-type animals with *daf-2* and *daf-2; daf-16* double mutant animals in normal osmotic conditions (50 mM NaCl) and assayed the response of their progeny to 500 mM NaCl. Approximately 60 percent of the progeny from the cross of wild-type males with *daf-2* mutant hermaphrodites hatched and developed at 500 mM NaCl (Fig. 2a). By contrast, the reciprocal cross of *daf-2* mutant males with wild-type hermaphrodites and the cross of wild-type males with *daf-2; daf-16* double mutant hermaphrodites did not produce any progeny that hatched and developed at 500 mM NaCl (Fig. 2a). These results demonstrate that like parental exposure to mild osmotic stress, reduced maternal insulin-like signalling can protect progeny from the effects of strong osmotic stress. Importantly, these observations also indicate that there is a previously undescribed link between maternal insulin-like signalling and progeny physiology.

Parental exposure to osmotic stress has been hypothesized to protect progeny from the effects of osmotic stress by increasing the deposition of glycerol from mothers into embryos, since embryos from parents exposed to 300 mM NaCl have more glycerol than embryos from animals grown at normal osmotic conditions (50 mM NaCl)[9] and glycerol is known to be protective against various environmental stresses[16,17]. We confirmed that exposure of parents to 300 mM NaCl resulted in progeny that are resistant to the effects of 500 mM NaCl (Fig 2b–c and Supplementary Table 3), and we discovered that the glycerol biosynthetic enzyme GPDH-2 is required for parental exposure to 300 mM NaCl to protect progeny from 500 mM NaCl (Fig. 2b) but does not affect the response to osmotic stress of animals with parents grown at 50 mM NaCl (Supplementary Fig. 3a). These observations are consistent with the hypothesis that an increased level of glycerol is required for adaptation to osmotic stress. However, it remained unclear how these observations relate to our finding that reduced maternal insulin-like signalling can protect progeny from strong osmotic stress (Fig. 2a), since previous studies indicated that embryos from *daf-2* mutant hermaphrodites contain the same amount of glycerol as embryos from wild-type animals[9] and hence that *daf-2* mutant mothers do not deposit more glycerol into embryos. We hypothesized that

reduced maternal insulin-like signalling protects progeny from the effects of osmotic stress not by increasing deposition of glycerol from mothers into embryos but rather by increasing glycerol production in embryos via GPDH-2. To test this hypothesis, we crossed *gpdh-2* mutant males with *daf-2; gpdh-2* double mutant hermaphrodites. GPDH-2 was required for reduced maternal insulin-like signalling to protect progeny from developmental arrest (Fig. 2d). To test if GPDH-2 functions maternally, we crossed wild-type males with *daf-2; gpdh-2* double mutant hermaphrodites. GPDH-2 was not required in mothers to protect progeny from developmental arrest in response to osmotic stress (Fig. 2d). We conclude that the inhibition of maternal insulin-like signalling does not result in the increased deposition of glycerol into embryos but rather results in increased glycerol production in embryos. Importantly, these results reveal that there is a heritable link between maternal insulin-like signalling and progeny metabolism.

We hypothesized that the heritable effects of maternal insulin-like signalling on progeny might be mediated by insulin-like signalling to the germline. To test this hypothesis, we expressed rescuing copies of the wild-type *daf-2* or *daf-16* genes specifically in the germline. Germline-specific expression of either *daf-2* or *daf-16* was sufficient to rescue the effects of deficient maternal insulin-like signalling on progeny response to osmotic stress (Fig. 2a). In addition, overexpression of *daf-2* in the germline blocked the protective effects of parental exposure to 300 mM NaCl on progeny response to 500 mM NaCl (Supplementary Fig. 3b). These data suggest that maternal exposure to 300 mM NaCl inhibits insulin-like signalling to the germline and that this loss of insulin-like signalling to the germline protects progeny from the effects of osmotic stress.

Insulin-like signalling to the *C. elegans* germline both inhibits DAF-16[18] and activates the RAS-ERK pathway[19], which includes the Raf protein LIN-45, the Mek protein MEK-2 and the Erk protein MPK-1[20]. We found that partial loss-of-function mutants in *lin-45, mek-2*, or *mpk-1* (null mutants are lethal) did not arrest development at 500 mM NaCl (Fig. 3a and Supplementary Fig. 3c). In addition, (*i*) treatment of wild-type animals with the MEK inhibitor U0126[21] or RNAi knockdown of *mek-2* prevented developmental arrest in response to 500 mM NaCl (Supplementary Fig. 3d–3f), (*ii*) RAS-ERK signalling functioned maternally to regulate progeny response to osmotic stress (Fig. 3b), (*iii*) GPDH-2 was required for reduced RAS-ERK signalling to protect animals from developmental arrest (Fig. 3a) and (*iv*) maternal exposure to osmotic stress inhibited MPK-1 activation in the germline (Fig. 3c and Supplementary Table 4), possibly by inhibiting the release of insulin-like peptides from sensory neurons (Supplementary Fig. 4a). We conclude that reduced insulin-like signalling from the soma (likely from sensory neurons) to the maternal germline protects progeny from the effects of osmotic stress by both activating DAF-16 and inactivating MPK-1.

To further examine how reduced maternal insulin-like signalling via the RAS-ERK pathway modifies progeny physiology we performed RNA-seq of wild-type and *lin-45* mutant embryos. We identified a total of 616 genes upregulated more than 2-fold and 1,310 genes downregulated more than 2-fold in *lin-45* mutant embryos (Supplementary Table 5). Among the 616 upregulated genes was *gpdh-2*, and we confirmed that *gpdh-2* mRNA expression is upregulated approximately 3-fold in *lin-45* mutant embryos using qRT-PCR (Fig. 3d). To

test if increased GPDH-2 expression results in increased glycerol production, we compared the glycerol-to-glucose ratios in wild-type and *lin-45* mutant embryos by mass spectrometry. We observed an 82% increase in the glycerol-to-glucose ratio in *lin-45* mutant embryos compared to that in wild-type embryos (Fig. 3e). These data are consistent with our hypothesis that reduced insulin-like signalling to the maternal germline protects progeny from the effects of osmotic stress by increasing the expression in embryos of the rate-limiting glycerol biosynthetic enzyme GPDH-2.

We note that previous studies found that adaptation to osmotic stress resulted in up to a 1,000% increase in glycerol in *C. elegans*[9], significantly higher than the increase we observed in *lin-45* mutants. Our genetic data demonstrate that GPDH-2 is required for reduced RAS-ERK signalling in the maternal germline to protect progeny from developmental arrest (Fig. 3a). However, given the modest increase in glycerol levels in *lin-45* mutants it remains possible, and perhaps likely, that one or more of the additional 615 genes upregulated in *lin-45* mutants also contribute to the resistance of these animals to developmental arrest in response to osmotic stress.

We tested whether parental exposure to other environmental stresses, such as bacterial infection or starvation, could similarly modify progeny response to environmental stress via RAS-ERK signalling. Exposure of *C. elegans* to the opportunistic pathogen *P. aeruginosa* PA14 slowed early larval development (Fig. 4a), parental exposure to PA14 enhanced this slowing of larval development (Fig. 4a), and this heritable slowing of larval development in response to bacterial infection required RAS-ERK signalling (Fig. 4a). However, maternal exposure to PA14 did not protect progeny from developmental arrest at 500 mM NaCl (Supplementary Fig. 4b) but rather resulted in progeny that were more sensitive to arrest in response to osmotic stress (Fig. 4b). In addition, we found that RAS-ERK signalling was required for L1 arrest in response to starvation (Fig. 4c). Collectively, these results suggest that maternal exposure to environmental stress modifies progeny physiology via RAS-ERK signalling, but that the effects of these stresses on progeny are different for different environmental stresses.

In conclusion, we propose a model in which the inhibition of insulin-like signalling to both the intestine and the germline can enhance *C. elegans* survival during osmotic stress but in which the effects of inhibition of insulin-like signalling to these two tissues are distinct. Specifically, the loss of insulin-like signalling to the intestine enhances resistance to osmotic stress by promoting developmental arrest, whereas the loss of insulin-like signalling to the maternal germline enhances progeny resistance to osmotic stress by increasing glycerol synthesis in embryos (Fig. 4d). In this model, information about the maternal environment is inherited via germ cells to enhance progeny resistance to future environmental stress.

The salt concentrations at which *C. elegans* arrests development in response to osmotic stress are approximately those of seawater, 480 mM Na^+ and 559 mM Cl^-[22]. We speculate that both the state of immobile arrested development and the ability of parents to confer progeny resistance to osmotic stress evolved to enhance organismal survival in response to osmotic stress caused by seawater. The insulin signalling pathway is broadly conserved among metazoa, and we postulate that insulin signalling to the germline plays a role in

several human developmental and metabolic abnormalities known to result from abnormal insulin signalling, such as intrauterine growth restriction (IUGR), obesity, and type-2 diabetes, all of which have been linked to maternal environmental stress[8, 23]. Consistent with this hypothesis, Huypens *et al.* (2016) recently reported that feeding parental mice a high-fat diet causes epigenetic changes to oocytes that results in progeny that are more susceptible to both obesity and diabetes[7]. These observations of a maternal high-fat diet modifying progeny physiology via oocytes in mice are similar to our observations of maternal exposure to osmotic stress modifying progeny physiology via oocytes in *C. elegans*. We propose that modified insulin-like signalling to the mouse germline might be the mechanism underlying these epigenetic changes in mouse oocytes.

Methods

Strains

All *C. elegans* strains were cultured as described previously[24] and maintained at 20°C unless noted otherwise. The Bristol strain N2 was the wild-type strain. Mutations used are:

LGI: *daf-16(mu86), mek-2(ku114), ins-18(ok1672), ins-18(ok2478), ins-26(tm1983), ins-28(ok2722), ins-29(tm1922), ins-30(ok2343), ins-33(tm2988), ins-36(tm6125), rrf-1(pk1417); gpdh-1 (ok1558)*

LGII: *ins-2(tm4467), ins-3(ok2488), ins-3(tm3608), ins-4(ok3534), ins-5(tm2560), ins-6(tm2416), ins-11(tm1053), ins-12(tm2918), ins-13(tm4856), ins-14(tm4886), ins-15(ok3444), ins-19(tm5155), ins-20(tm5634), ins-31(ok3543), ins-32(tm6109), ins-37(tm6268), age-1(hx546), knuSi379 [Pmex-5::daf-16cDNA::GFP::nos-2 3′ UTR,unc-119(+)]*

LGIII: *daf-2(e1370), mpk-1(n5639), ins-17(tm790), ins-21(tm5180), ins-22(tm4639); gpdh-2(ok1733)*

LGIV: *lin-45(n2018), lin-45(n2506), ins-1(nj32), ins-7(tm1907), ins-8(tm4144), ins-34(tm3095); unc-31(ft1); zIs356 (daf-16::GFP); pmk-1(km25); skn-1(zu67)*

LGV: *daf-28(tm2308), ins-10(tm3498), ins-27(ok2474), ins-35(ok3297), him-5(e1490)*

LGX: *ins-9(tm3618), pdk-1(sa709) nIs349[Pceh-28::4xNLS::mCherry; lin-15(+)];*

unknown linkage: *vizIs23 [pie-1p::GFP::daf-2(WT)::pie-1 3′UTR + unc-119(+)], vizIs22 [pie-1p::GFP::daf-2(WT)::pie-1 3′UTR + unc-119(+)], nIs343 [Pegl-1::4xNLS::GFP; lin15(+)]; otIs39[unc-47p::GFP];*

Extrachromosomal arrays: *naEx187[pGC467 (ins-3(+)), pRF4)rol-6(su1006)], hpEx2906 [Prgef-1::daf-2; Pmyo-2::mCherry], hpEx3369 [Pges-1::daf-2; Pmyo-2::mCherry], hpEx2905 [Pmyo-3::daf-2; Pmyo-2::mCherry] fx325 [Posm-6::unc-31a; Pmyo-3::mCherry; unc-119(+)]*

Assay for developmental arrest

Approximately 200 developing eggs from mothers grown at 50 mM NaCl (unless otherwise noted) were collected and placed on standard NGM plates containing varying concentrations of NaCl at 25°C for 48 hrs. After 48 hrs, animals that remained immobile and were not feeding were scored as arrested. Mobile animals that were feeding were scored as developing. %L2+ is defined by the percent of animals mobile and feeding and that have developed past the L1 larval stage. %Failing to arrest is defined by the percent of animals mobile and feeding (unlike animals normally arrested in response to osmotic stress) but includes L1 stage larvae.

Assay for survival after arrest

Approximately 100 developing eggs from mothers grown at 50 mM NaCl were collected and placed on standard NGM plates containing varying concentrations of NaCl at 20°C for 24 hrs. After 24 hrs arrested animals were picked onto plates containing 50 mM NaCl and allowed to recover for 24 hrs. After 24 hrs the fraction that regained mobility and resumed development were scored as surviving and the fraction that failed to resume development and did not respond to touch were assumed to be dead.

DAF-16::GFP localization

Confocal microscopy was performed using a Zeiss LSM 800 instrument. The resulting images were prepared using ImageJ software (National Institutes of Health). Image acquisition settings were calibrated to minimize the number of saturated pixels and were kept constant throughout the experiment.

mRNA expression analysis by RNAseq and qRT-PCR

L4-stage wild-type and *lin-45* animals were placed on standard NGM plates containing either 50 mM or 300 mM NaCl for 24 hrs. Developing eggs from these animals were collected and placed at either 50 mM or 500 mM NaCl for 6 hrs. After 6 hrs embryos were collected in M9, and RNA was extracted using TissueRuptor and the RNeasy Mini kit (QIAGEN). For RNAseq, RNA integrity and concentration were checked on a Fragment Analyzer (Advanced Analytical). The mRNA was purified by polyA-tail enrichment, fragmented, and reverse transcribed into cDNA (Illumina TruSeq). cDNA samples were then end-repaired and adaptor-ligated using the SPRI-works Fragment Library System I (Beckman Coulter Genomics) and indexed during amplification. Libraries were quantified using the Fragment Analyzer (Advanced Analytical) and qPCR before being loaded for paired-end sequencing using the Illumina NextSeq. For qRT-PCR reverse transcription was performed using SuperScript III (Invitrogen), and quantitative PCR was performed using Applied Biosystems Real-Time PCR Instruments. All results are normalized to the mRNA levels of histone gene *his-24*.

Primers

gpdh-2

forward: tttgatccaaccgtccgtat reverse: cgaattgatgtggaacaacg

his-24

forward: atgatcaaggaggccatcaa reverse: tgagcattgatctggatgaca

Assay for wild-type adaptation to osmotic stress

Wild-type embryos were placed on standard NGM plates containing 300 mM NaCl at 20°C for 60 hrs and allowed to develop to adulthood. Embryos from these adult animals were extracted using a razor blade into M9 solution and pipetted onto plates containing 500 mM NaCl. These animals were allowed to develop for 48 hrs at 20°C. % failing to arrest is defined by the fraction of animals that were mobile and feeding.

Cross progeny analysis

Approximately 20 L4 hermaphrodites were crossed with wild-type or *daf-2(e1370)* males for 24 hrs at 25°C. After 24 hrs, embryos were dissected from hermaphrodites using a razor blade and then placed on plates containing 500 mM NaCl at 20°C for 24 hrs. %L2+, the percentage of animals that developed past the L1-larval stage. All males contained *nIs343 (Pegl-1::*4xNLS::mCherry*); nIs349 (Pceh-28::*4xNLS::mCherry) for the identification of cross progeny.

Photographs

Photographs of animals and embryos on standard NGM plates containing either 300 mM or 500 mM NaCl were obtained using an AxioCam MRm camera (Zeiss).

dpMPK-1 imaging

Wild-type animals at the L4 stage were placed on either 50 mM or 300 mM NaCl NGM plates seeded with OP50 at 20°C for 24 hrs, and germlines were extruded. Dissections were performed as described previously.[25–26] Briefly, dissections were performed within 5 min of adding levamisole to achieve optimal diphosphorylated MPK-1 (dpMPK-1) staining. The dissected germlines were then fixed in 3% paraformaldehyde for 10 min, followed by a post-fix in 100% methanol at −20°C. The fixed germ lines were then processed for immunoflourescence staining as described[27–29]. anti-MAP Kinase was used at a dilution of 1:200 (Clone MAPK-YT, Sigma, St. Louis, MO). Secondary antibodies were donkey anti-mouse Alexa Fluor 594 and used at a dilution of 1:400. Each gonad was photographed as a montage, with each image taken as a 0.15 μ section and captured with overlapping cell boundaries at 63× magnification. Images were taken using a Zeiss Axio Imager upright microscope with AxioVs40 V4.8.2.0 microimaging software and an Axio MRm camera (Zeiss). Montages were then assembled using Adobe Photoshop CS5.1, and white levels were uniformly adjusted using Affinity Illustrator to reduce background.

RNAseq prep and data analysis

For supplemental tables 1 and 3 RNA integrity and concentration were checked on a Fragment Analyzer (Advanced Analytical). The mRNA was purified by polyA-tail enrichment, fragmented, and reverse transcribed into cDNA (Illumina TruSeq). cDNA samples were then end-repaired and adaptor-ligated using the SPRI-works Fragment Library System I (Beckman Coulter Genomics) and indexed during amplification. Libraries were

quantified using the Fragment Analyzer (Advanced Analytical) and qPCR before being loaded for paired-end sequencing using the Illumina NextSeq. For QC purposes, BEDTools[30] (version: 2.25.0) was used to count the reads falling into genes, coding regions, intronic regions, 5′ or 3′ UTRs, flanking 3-kb genic regions and intergenic regions. Other basic statistics, including mapping rate, ratio of sense vs. anti-sense reads and rRNA percentages were also collected for each sample. The reads were first cleaned up by removing reads aligned to rRNAs (BWA 0.6.1[31] and BEDTools 2.25.0 were used). RSEM[32] (version 1.2.15) was used to estimate gene levels based on ce10 ensembl annotations downloaded from UCSC genome table browser[33]. The gene expression count table was then imported into DESeq[33] (version 1.10.1) for differential gene expression test. See README tab in supplemental table 2 for detailed descriptions of RNA-seq and analysis for supplemental table 2.

Pathogen exposure and development assay

Wild-type embryos were placed onto either NGM plates seeded with *E. coli* OP50 or slow-killing assay plates seeded with *P. aeruginosa* PA14 and allowed to grow at 25C for 72 hrs. After 72 hrs embryos were collected from adults and placed on new plates seeded with either OP50 or PA14 and placed at 25C.

M-cell division in response to starvation

M-cell division analysis was performed as described previously[10].

Statistics and Reproducibility

ANOVA analysis with post hoc p-value calculations were used for Fig. 2, Fig. 3a, Fig. 3b, Fig. 4a, Supplemental Fig. 5 and Supplemental Fig. 8. Unpaired two-tail students t-test was used for Fig. 1, Fig. 3d, Fig. 3e, Fig. 4b, Fig. 4c, Supplemental Fig. 1, Supplemental Fig. 3, Supplemental Fig. 6 and Supplemental Fig. 9 * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** $p < 0.0001$. No statistical method was used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Metabolite preparation for quantification

Approximately 100 ul of concentrated embryos were collected by egg prep and placed on normal NGM agar plates for 3 hrs to recover. After 3 hrs embryos were collected in M9, pelleted, and frozen. Frozen embryos were resuspended in 400 ul PBS and homogenized by douncing. Homogenized embryos were centrifuged at 3000 RPM for 2 min to remove undounced tissue and 200 ul of supernatant was mixed with 800 ul of methanol and dried to extract polar metabolites. Dried samples were stored at −80° C.

Metabolite profiling

Liquid chromatography and mass spectrometry were performed as described previously[33].

Assay for L2, L3 and L4 stage developmental arrest

Approximately 50 L2, L3 or L4-stage animals were placed onto standard NGM Petri plates containing 500 mM NaCl for 24 hrs. After 24 hrs animals that were immobile and not developing or responding to touch were moved to plates containing 50 mM NaCl. Percent developing, percent of animals that resumed development and mobility after returning to normal growth conditions.

MEK inhibitor exposure

The MEK inhibitor U0126 (U120 Sigma-Aldrich) was resuspended in DMSO and added to standard NGM Petri plates at a final concentration of 100 uM. These plates were then seeded with *Escherichia coli* OP50. Wild-type embryos were placed on plates containing either DMSO alone or DMSO and the MEK inhibitor U0126. Animals were allowed to grow for 72 hrs at 25°C. After 72 hrs, embryos from adult animals were collected and placed onto Petri plates containing 500 mM NaCl at 25°C for 24 hrs and the fraction of animals mobile and developing were counted.

mek-2 RNAi exposure

Wild-type embryos fed *Escherichia coli* HT115 containing either the empty vector L4440 or a *mek-2* RNAi vector (Ahringer library - Source Biosciences) were grown at 20°C for 72 hrs. After 72 hrs, embryos were collected and placed onto Petri plates containing 500 mM NaCl for 24 hrs at 25°C and the fraction of animals mobile and developing were counted.

Assay for *daf-2* adaptation to osmotic stress

L4 animals were placed on standard NGM plates containing 300 mM NaCl at 25°C overnight. Embryos from resulting adult animals were extracted with a razor blade into M9 solution and pipetted onto plates containing 500 mM NaCl. These animals were allowed to develop for 48 hrs at 20°C. %L2+, the percentage of animals that developed past the L1-larval stage.

Data availability

RNA-seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession code GSE91073 and GSE91039. Data supporting the findings of Fig. 1b, 1c, 1d, 2a, 2b, 2d, 3a, 3b, 3d, 3e, 4a, 4b, 4c and Suppl. Fig. 1b, 1c, 1d, 1e, 1f, 1g, 3b, 3d, 3e, 3f, 4b, 4c, and 4d are provided in Supplementary Table 6. All other relevant data are available from the authors on reasonable request and/or are included with the manuscript.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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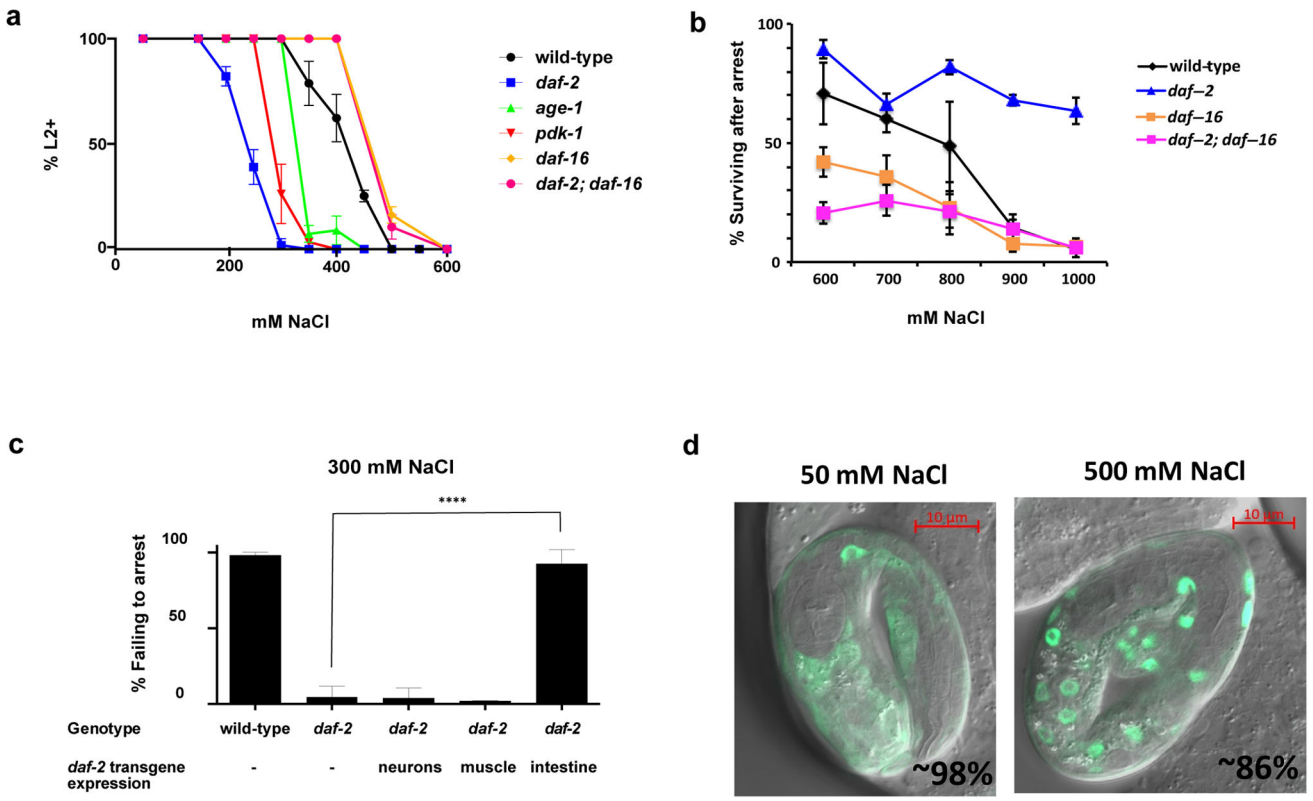

Figure 1.

Insulin-like signalling to the intestine regulates developmental arrest in response to osmotic stress. (a) Percent of wild-type, *daf-2(e1370), age-1 (hx546), pdk-1(sa709), daf-16(mu86)* and *daf-2(e1370); daf-16(mu86)* animals developing past the L1 larval stage after 48 hrs. Error bars, s.d. $n = 3$ experiments of >100 animals $P < 0.01$ for all genotypes (two-tailed t-test) (b) Percent of wild-type, *daf-2(e1370), daf-16(mu86)* and *daf-2(e1370); daf-16(mu86)* animals that resume development after 24 hours of exposure to osmotic stress. Error bars, s.d. $n = 3$ see Supplementary Table 6$P < 0.01$ for all genotypes (two-tailed t-test) (c) Percent of wild-type and *daf-2(e1370)* animals developing past the L1 larval stage at 300 mM NaCl after 48 hrs. Neuron-specific expression was driven by *Prgef-1*; intestine-specific expression was driven by *Pges-1*; muscle-specific expression was driven by *Pmyo-3*. Error bars, s.d. $n =$ 3 experiments of >100 animals (d) Confocal images of DAF-16::GFP after 6 hrs of exposure to 50 mM and 500 mM NaCl. Scale bar 10 µm. 3 experimental replicates of >100 animals. The quantified results are presented as mean ± s.d. using two-tailed *t*-test ****$P < 0.0001$ were considered significant. n.s., not significant. See Statistics Source Data in Supplementary Table 6

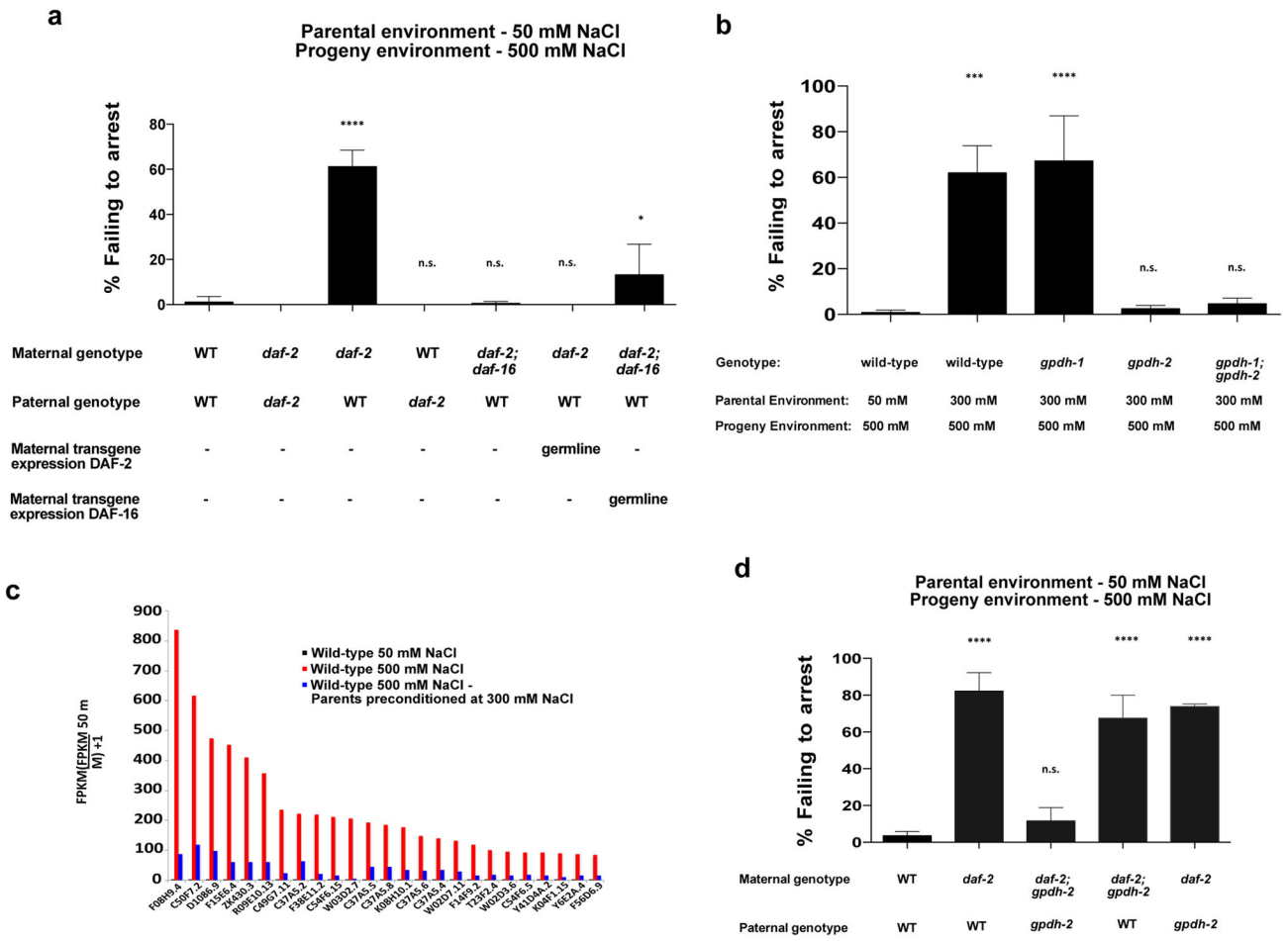

Figure 2.

Insulin-like signalling to the maternal germline regulates progeny response to osmotic stress (a) Percent of wild-type, *daf-2(e1370)* and *daf-2(e1370); daf-16(mu86)* cross progeny failing to arrest development after 48 hrs at 500 mM NaCl. Males contained *(Pegl-1::*4xNLS::GFP*); him-5(e1490); nIs349 (Pceh-28::*4xNLS::mCherry) for the identification of cross progeny. The *pie-1* promoter was used to drive germline specific expression of DAF-2 and the *mex-5* promoter was used to drive germline specific expression of DAF-16. Error bars, s.d. $n = 7$, 3, 6, 3, 3, 3, and 3 see Supplementary Table 6. (b) Percent of wild-type, *gpdh-1(ok1558)*, and *gpdh-2(ok1733)* animals failing to arrest development at 500 mM NaCl after 48 hrs. Error bars, s.d. $n = 3$ experiments of >100 animals (c) Average fold change of 2 replicates of the 25 most upregulated genes in embryos in response to osmotic stress after 6 hrs. (d) Percent of wild-type, *daf-2(e1370)* and *gpdh-2(ok1733)* cross progeny failing to arrest development after 48 hrs at 500 mM NaCl. Males contained *otIs39 (Punc-47::GFP); him-5(e1490)* for the identification of cross progeny. Error bars, s.d. $n = 3$ experiments of >20 animals. The quantified results are presented as mean ± s.d. using ANOVA. $^{*}P < 0.05$, $^{***}P < 0.001$, **** $P < 0.0001$ were considered significant. n.s., not significant. See Statistics Source Data in Supplementary Table 6

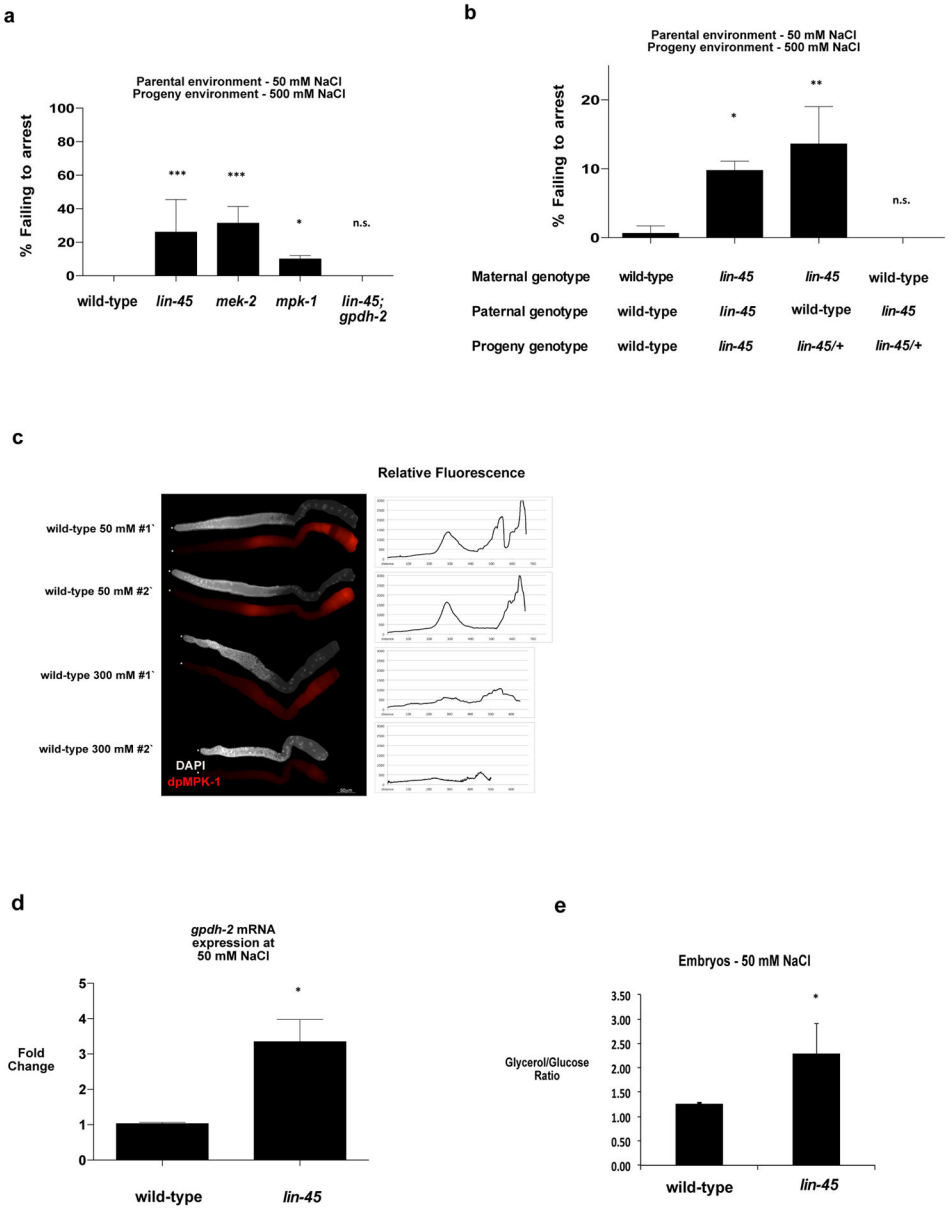

Figure 3.

Insulin-like signalling to the maternal germline modifies progeny response to osmotic stress by regulating the RAS-ERK-like pathway. (a) Percent of wild-type and *lin-45(n2018), mek-2(ku114)*, *mpk-1(n5639)* and *lin-45(n2018); gpdh-2(ok1733)* animals failing to arrest development at 500 mM NaCl after 48 hrs. Error bars, s.d. $n = 3$ experiments of >100 animals (b) Percent of wild-type and *lin-45(n2018)* cross progeny failing to arrest development at 500 mM NaCl after 48 hrs. Males contained *otIs39 (Punc-47::GFP); him-5(e1490)* for the identification of cross progeny. Error bars, s.d. $n = 3$ experiments of >20 animals. (c) Representative germlines dissected from wild-type animals exposed to either 50 mM NaCl or 300 mM NaCl and stained for DNA (DAPI, white) and diphosphorylated MPK-1 (dpMPK-1) (red) Each condition was replicated 16 times. Scale bar 50 μm (d) Relative expression of *gpdh-2* mRNA in wild-type and *lin-45(n2018)* embryos

measured by qRT-PCR and normalized to the expression of the histone *his-24.* Error bars, s.d. $n = 3$ experiments from pellets of >1000 embryos (e) Glycerol-to-glucose ratio in wild-type and *lin-45(n2018)* mutant embryos. Error bars, s.d. $n = 3$ experiments from pellets of >1000 embryos. The quantified results are presented as mean ± s.d. using ANOVA (a, b) and two-tailed t-test (d, e). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ were considered significant. n.s., not significant. See Statistics Source Data in Supplementary Table 6

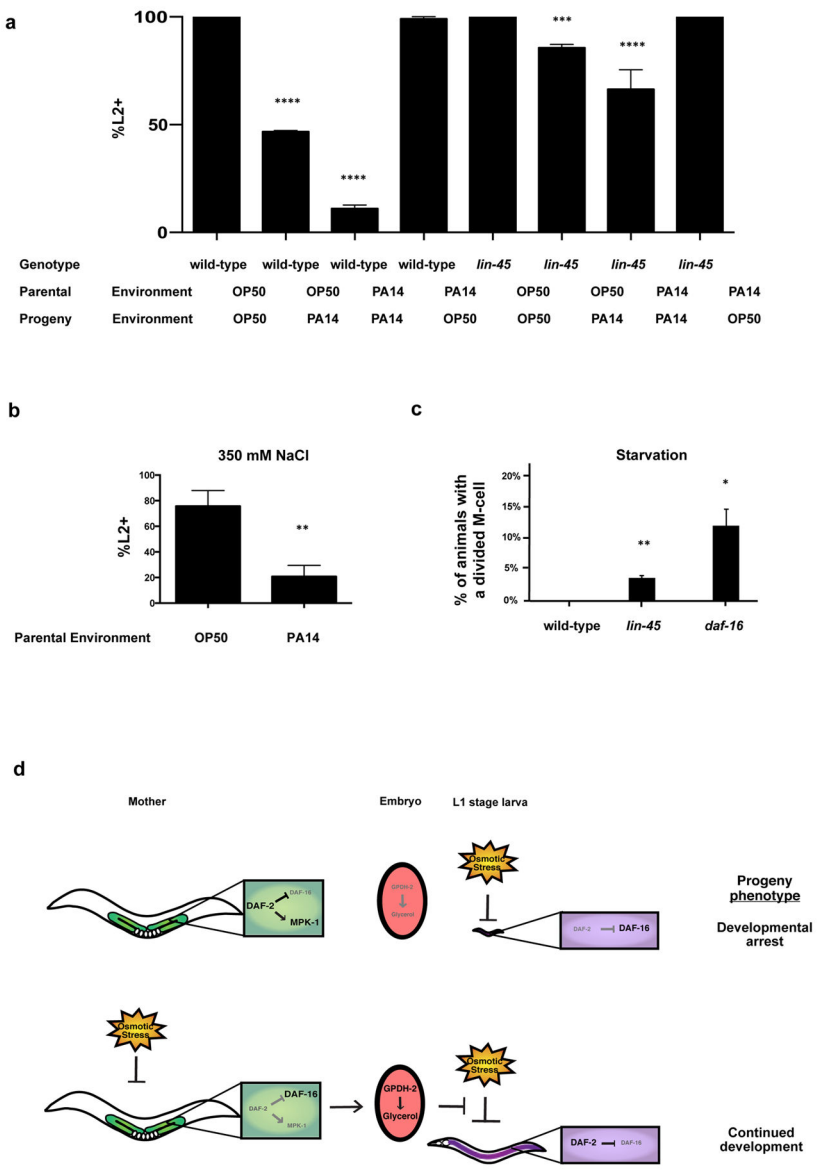

Figure 4.

RAS-ERK signalling regulates *C. elegans* response to bacterial infection and starvation (a) Percent of animals expressing *lin-4::YFP* after 24 hrs of exposure to either *E. coli* OP50 or *P. aeruginosa* PA14. Error bars, s.d. $n = 3$ experiments of >100 animals (b) Percent of animals failing to arrest development after 24 hrs at 350 mM NaCl. Error bars, s.d. $n = 3$ experiments of >100 animals (c) Percent of wild-type, *lin-45(n2018)* and *daf-16(mu86)* mutants with a divided M-cell after 7 days without food in S-basal at 20°C Error bars, s.e.m. $n = 4$ experiments of >100 animals (d) Model for how maternal exposure to osmotic stress inhibits DAF-2 activity in the germline and affects progeny response to osmotic stress. See text for details. Color code: Red, embryo; green, germline; purple, intestine. The quantified results are presented as mean ± s.d. (a, b) and s.e.m (c) using ANOVA (a) and two-tailed t-test (b,c).

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ were considered significant. n.s., not significant. See Statistics Source Data in Supplementary Table 6